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AGRICULTURE AND THE WAR MACHINE.

Sir E. J. RUSSELL, F.R.S.,

Director, Rothamsted Experimental Station.

THROUGH all the ages agriculture and war have been regarded as mutually antagonistic; it has been left to this generation to fuse them and make agriculture simply a part of the war machine. We have only recently begun to do this: the Germans, on the other hand, were already on the way some ten years ago and so have a considerable start over us. We have, however, some advantages which they lack.

In this country the farmer's peace-time aim was to keep his farm going, often no easy task. He produced whatever in his judgment seemed most likely to achieve this end, using whatever materials suited him best, regardless of where they came from. He was under no sort of restriction, and could accept or reject any advice given to him. Large quantities of fertilisers and feeding-stuffs were imported from many different countries, quite regardless of whether or not they could have been produced in this country. Many farmers frankly did little more than "processing": they imported feeding-stuffs from overseas and converted them into meat and milk.

German agriculture, on the other hand, has for some years past been very carefully organised to make the nation as nearly self-sufficient as possible. The German farmer had no need to worry about his own position: prices were fixed by the State at a satisfactory level, and labour could not easily move away from the land; all that had to be done was to obey orders and produce the required foods, but he had to be as far as possible self-sufficient and independent of imported materials. He was shown how to do this.¹ The whole German nation was put on to the dietary that the farmers could produce, and as a result the Germans

¹ In spite of all the organisation before the war German agriculture had been moving in the same direction as British agriculture, though to a much less extent. The area of arable land was shrinking and the grass was increasing; livestock were forming a larger part, and arable crops a smaller part of the total output; and until stronger measures were taken the number of agricultural workers was diminishing.

have much less change to make in their food and their farming than we have.

Our peace-time dietary was much richer and more varied than the German. We were far from being self-sufficient, indeed only 40 per cent., reckoned in money value, of our food was produced here, and 60 per cent. was imported. Worse still, so far as war is concerned, the 40 per cent. home production was not evenly spread over the staple foods. We produced roughly about 10 per cent. of our butter, 20 per cent. of our wheat and flour, 30 per cent. of our cheese, and 50 per cent. of our meat; but nearly all of our oats and potatoes and all of our liquid milk. Our imported foods and drinks came from almost every part of the civilised world. If you went into a small village shop and noted the country of origin of each of the foodstuffs you would have needed a world atlas to place them all.

Our dietary, like our agriculture, presupposed peace and a highly organised and delicately adjusted international trade. It was, in fact, rather extravagant in its demand on the land. About 2 acres were needed on the average to produce the food of one head of population. This, however, included a good deal of land in overseas countries where the yields are lower than ours: at British levels of yield the area of land needed was about 1·6-1·7 acres per head; *i.e.*, to feed 10 people some 17 acres were necessary.¹

The German peace-time dietary, on the other hand, was much simpler than ours: it included only little that had to be imported, and it made much less demand on the land than ours did. At British levels of yield (which are not very different from the German) about 1 acre per head was needed just before the war, *i.e.*, 17 acres would feed, not 10 persons as here, but 17. Had we adopted the German dietary in peace-time we could have produced not 40 but 70 per cent. of our food, and it would have been fairly easy to push up the yields to make this figure 80 per cent. Theirs was not an interesting dietary: compared with ours there was twice as much potatoes, only half as much sugar, less butter, little lamb or mutton, and only half as much beef, and that not well finished. Two years before the war a well-placed individual, who could certainly get whatever was to be had, told me, as I was crossing Germany, of his "yearning" for the beautiful quality beef he had been able to get in London but not in Germany. I

¹ Two separate estimates can be made: (a) The population of Great Britain in peace-time was about 46 millions: 40 per cent. of the food for these corresponds to the feeding of 18½ millions. We had 32 million acres of cultivated land: this works out to nearly 18 acres per 10 persons. (b) The areas of land which at average yields would give the quantities of the different foods needed per head of population are set out in Table 1. Official estimates are available for the yields of arable crops, but we have to assume values for yields of meat and milk. This gives 16 acres per 10 persons,

told him that it was still produced in these Islands, and there was no good reason why he should not have as much as he wanted—but he shook his head sadly.

The two dietaries are set out in Table I. along with an estimate of the areas of land needed on average British yields for arable crops and, in absence of statistics, assumed yields for meat and milk.¹

TABLE I.

	Food Consumption Pounds per head per annum (P. Lamartine Yates).		Land required for produc- tion at British levels of yield : acres.	
	Great Britain.	Germany.	Great Britain.	Germany.
Bread and Flour	197	222	0·15	0·16
Potatoes	210	398	0·05	0·03
Sugar	109	56	0·04	0·02
Beef and Veal	66	34	0·60	0·31
Pork	48	65	0·18	0·25
Mutton and other meat	29	—	0·26	—
(All meat)	143	100	1·04	0·56
Milk (gall.)	20	21	0·06	0·06
Butter	22	16·4	0·18	0·14
Margarine	8	15·5	—	—
Cheese	9·5	12·6	0·02	0·03
Eggs (No.)	153	126	—	—
			1·50	1·00
Other foods			0·1	·05
			1·60	1·05

Fish, fruit and vegetables are not included in the above table; the figures for Great Britain in 1934 were, in lb. per head:—Fish, about 40-45; Vegetables, 100; Fruit, 115. The German consumption of vegetables and fruit was lower, but, like ours, was expanding.

This great difference in the dietaries of the two nations caused wide differences in the agriculture, but made German farming much simpler than ours. The data have been collected in a valuable book by P. Lamartine Yates.² Far more of their land is under the plough than here—68 per cent. as against 41 per cent. in Great Britain: much of it is worked on a three-course rotation, corn—corn

¹ 110 lb. meat (220 lb. live weight) per acre for beef and mutton. 300 gallons milk per acre. Pork—from arable land: 7 lb. barley = 1 lb. pork.

² P. Lamartine Yates. *Food Production in Western Europe*. Longmans, 1940.

—roots; or a six-course, corn—corn—roots—corn—corn—grass, and the commonest corn crop is rye, the easiest of all to grow. Much more labour is employed per 100 acres, but it is less efficient than ours. In Great Britain one agricultural worker (including the farmer) feeds about 17 “persons”¹ and has a net output of about £200 per annum. The German agricultural worker feeds 7 “persons” and has a net output of only £70 per annum. There are correspondingly big differences in the numbers of livestock per worker, and the numbers of workers per 100 acres of cultivated land. It cannot be too clearly stated that the British agricultural worker has a higher value of output per annum than any other agricultural worker in Europe. With all the high rate of labour on the farm, yields are but little higher than in this country, although in consequence of the higher proportion of arable land the average value of output per acre is somewhat higher—£8 in place of our £6 at equal price levels (Table II.).

TABLE II.

	Agricultural Output 1937 (P. Lamartine Yates)						
	Output per worker		Weekly wages per hired worker (shillings)	Acres per worker	Stock units per worker	Output per acre*	
	Gross	Net				Gross	Net
	£	£				£	£
Great Britain	240	200	30-36	33·8	10·3	7	6
Denmark -	180	155	23-26	15·7	8·4	11	10
Netherlands -	150	120	23-30	9·0	4·9	17	14
Belgium -	110	100	18-22	7·4	3·4	15	14
Switzerland -	110	100	27-20	7·1	4·3	17	15
France -	90	90	20-28	11·6	2·8	8	8
Germany -	70	70	18-23	7·0	2·8	8	8

* Rough grazings in Great Britain reckoned at half their acreage, and Alpine grazings in Switzerland at one quarter.

The German system needs no modification for war conditions; it is, in fact, an admirable war-time system. The difficulty of labour is completely overcome by collecting from the occupied countries all the men and women desired; questions of wages, conditions of life and of work do not arise, and, as the labour never had been, and from the nature of the system never need be, very efficient, the lack of training and experience is of no great moment. How long can the system last? That remains to be seen, but we must expect that the iron heel on the farm, and the taking of food from occupied countries, will keep Germany sufficiently fed for a long time to come.

The small proportion of grass and of roots in Germany is explained by the smaller importance of livestock in German than in British agriculture. Nevertheless, the output of milk and pork

¹ O. J. Beilby. *Empire Jl. Expt. Agric.* 1941, Vol. 9, pp. 137-144.

had to be maintained, and the Germans showed great ingenuity in providing home-grown feeding-stuffs. Twelve years ago, before the present programme was developed, they used to import some 30 per cent. of their total concentrates; but this was rapidly dropped to less than 10 per cent.; we, on the other hand, imported about 66 per cent. This drop in Germany was brought about in several ways. Ensilage was developed, portable outfits for steaming potatoes for livestock were put in circulation, fodder crops were extended and the grassland improved. A system of pig-feeding was worked out which reduced to a minimum the grain required, and made full use of home-grown fodder. The Germans are less likely to have to reduce total numbers of livestock than we are.

The difference in position of livestock in German and in British agriculture affects the manuring of the crops; in Great Britain most of our nitrogen and potash (though not phosphate) is supplied from farmyard manure, but in Germany more has to come from artificial fertilisers. That means a higher consumption of fertilisers than here, which, however, presents no difficulty. Nitrogenous fertilisers are made from the air, and so long as the factories remain at work the output can be maintained. Potash is almost entirely in German hands; they always had the chief deposits, and in addition now control the Alsatian and the Polish mines. Phosphate supplies caused them difficulty in the last war, but perhaps less in this; the chief source is French North Africa, but in the last 20 years other sources have been opened up. The conversion of insoluble mineral into soluble phosphate could not easily be done in the last war, but methods have in the meantime been developed.

The more closely one compares German and British agriculture the more it appears that German agriculture has for some years been an integral part of the war machine and, therefore, need now suffer little change, while ours has not, and we have to make considerable changes now while the war is on us, just as we did in the last war. In some respects our position is better now than it was then. Farmers and farm workers are much better organised than they were so that changes can be made far more quickly. The War Agricultural Committees are not new people, and their officers have usually already held posts as organisers, and know their counties and their farmers well. The research and advisory services are in full working order; problems as they arise can be dealt with, even if a perfect solution cannot always be found. It was, of course, unfortunate that the problems raised in the last war were not satisfactorily solved in the years of peace; we are once again back at some of them:—control of wireworms, manurial value of town refuse, sewage sludge and other wastes, utilisation of mineral phosphate, of straw, etc., all of which had

been set aside in 1919 for problems that then became more urgent.

We cannot hope to maintain our peace-time dietary during the war, and the changes must be considerably more drastic than any needed in Germany. Our consumption of meat, sugar, eggs and cheese must be cut down, and we must provide for an increased consumption of bread, potatoes and vegetables. Fortunately our people are taking the change in dietary very much better than might have been expected, in view of British conservatism in the matter of food, and fortunately also the new dietary seems to be as wholesome and as sustaining as the old one, thanks to the care taken in rationing, advice about cooking and the establishment of canteens.

The change in dietary involves corresponding changes in our farming:—

- (1) A large increase in production of cereals, potatoes and sugar beet, all wanted for human food;
- (2) Full maintenance and, if possible, increase of milk, in spite of the fact that some of the imported foodstuffs are cut off;
- (3) As high an output as possible of meat and eggs as long as this does not hamper (1) and (2);
- (4) An increased output of vegetables and fruit.

The Increase in Output.

I. OUR ENERGY SUPPLIES.

Cereals.—Wheat and, to a less extent, oats furnish the major part of our energy requirements. In peace-time we produced only about one-quarter of our total consumption of wheat, and a fair amount went to poultry, so that less than a quarter of the human consumption was provided from home sources. On English farms the output of wheat will increase considerably during the war, and this is a great comfort in view of the supreme importance of having unlimited bread supplies. Fortunately, also, wheat is one of the easiest of all foods to transport, and ample supplies are in Canada, the shortest of the long sea voyages.

Oats are in a rather special position. Thanks to an effective publicity campaign the consumption of oatmeal seems to be increasing in England, indeed in some places the housewives' enthusiasm ran ahead of the shopkeepers' supplies. This development is wholly beneficial, and those farmers who can grow good quality oats should increase their output in every way possible.

In the old days rye was often used for human food, and then it was given up in this country, although much used in Germany. More recently, however, increasing use is being made of rye for special foods. The grain is grown on contract, and I am informed that no less than 6,500 acres are wanted this season.

Potatoes.—These constitute the second most important source of our energy requirements and, indeed, they give a larger energy return per acre than any other crop. An acre of potatoes provides approximately twice as many calories as an acre of wheat or oats.¹ Potatoes are in the special position that in peace-time we satisfied almost the whole of our requirements, importing as a rule only a certain number of earlies. We have therefore no leeway to make up here. But our peace-time annual consumption was only about 200 lb. per head as against 400 lb. in Germany, and the curtailing of meat, fish and eggs should lead to a larger consumption. Hence the drive for a larger potato output in gardens and allotments, and on farms. Certain conditions must, however, be fulfilled. The seed must be both vigorous and free from disease. There is a real danger in extending the cultivation of potatoes that its diseases also are spread, notably the virus diseases, unless special care is taken to ensure healthy seed, and yet the cultivation of potatoes must be much more widely spread. In peace-time it is one of the most local of all our crops, being confined largely to the eastern part of England and Scotland, and to certain restricted areas in the west. In war-time transport cannot be spared, and there should be a much larger measure of local self-sufficiency. As it is quite impossible to foretell the yield, this increased acreage may result in crops in excess of what the markets would normally take. In peace-time this risk was a powerful deterrent against extending too much the potato acreage; in war-time not only must risk be taken, but one must hope that the bumper crop will come. The financial risk is borne by the Government, but the utilisation of excess potatoes is one of the most urgent of war-time problems. More human consumption can be encouraged by the development of special methods of treatment, such as chips, crisps, etc.; above all by suitable propaganda in the towns, establishment of potato bars, etc. Potatoes can also be used for the manufacture of farina or other foodstuff, or as food for livestock, especially if travelling cooks could be sent round. The problem should be taken in hand before the crop is ready.

¹ Wheat gives flour furnishing about 2.6 million calories per acre at 17.7 cwt. yield, less 1.4 cwt. per seed, and 85 per cent. extraction for flour; while potatoes at 6.5 tons per acre yield, less 0.8 tons for seed and allowing for waste in peeling, give 5 million calories.

II. OUR PROTEIN SUPPLIES.

Protein for ourselves.—In the last war it was commonly stated that a reasonable dietary supplying enough energy would almost always supply enough nitrogen also. This may be so, but, unfortunately, the nitrogen in grains, *e.g.* wheat, oats, beans, etc., has not the same biological value as the nitrogen in animal products, meat, fish, milk and eggs. It is significant that in peacetime the British and American peoples eat more animal protein than most other races, and it seems reasonable to attribute a good deal of our driving force to that circumstance.

The proteins of green leaves seem to be in a different category, and some of them appear to have distinctly higher biological value than those of seeds. Green leaves, however, are not a good source of protein for human beings because of their large content of cellulose and fibre, which we cannot digest directly, although micro-organisms in the intestine can bring about decomposition. If it should become necessary, it would almost certainly be possible to separate the protein from these less useful constituents and make it available as human food. For the present most of the first-class protein comes from animal sources, and it must be admitted that there is ground for uneasiness on this account.

We started the war with a large population of livestock, in some ways larger than ever before, but the restriction on imported feeding-stuffs has compelled reduction; we have also reduced our import of finished animal foods—meat, eggs, cheese, etc. More labour and more skill are required in the production of animal than of vegetable protein. For many reasons, therefore, our supplies of high-class animal protein will tend to go down. Yet it is difficult to see how hard manual work can be done without it. Special arrangements have been made to supply additional cheese to agricultural workers and miners, and canteens make possible supplies for other workers. It is, however, up to the farmer to increase the output in every way possible: the power of endurance of our workers may still turn on this factor.

Protein for livestock.—While animals can transform second-class plant proteins into first-class ones they cannot make protein out of simple substances: only plants can do this; they alone have the remarkable power of building up proteins out of simple compounds like ammonia or nitrate. Kale is outstanding in this way, and is well ahead of most other crops. The numbers of pounds of protein equivalent and of starch equivalent made from 1 cwt. of sulphate of ammonia are on an average:—

	Kale	Oats	Swedes	Potatoes	Meadow hay
		Grain	Straw		
Protein equivalent	44	21	6	13	26
Starch equivalent -	302	168	114	403	174

It is certain that we do not use anything like enough sulphate of ammonia. In peace-time "Safety First" may be a justifiable rule; but in war-time there can be no fear of overproduction, and probably most of our crops would bear another cwt. per acre of sulphate of ammonia. In regard to other fertilisers the restricted supplies of potash and phosphate have made soil analysis more than usually important so as to ensure that these shall be used to the best advantage.

On most farms, however, the chief source of protein, or more strictly protein equivalent, for livestock is grass. Dr Norman Wright estimates that of the total of 3·7 million tons of protein equivalent consumed annually by the livestock of the United Kingdom in the years before the war, no less than 2·1 million tons came from temporary and permanent grass, 1 million was imported and 0·6 million came from home-grown cereals, roots, straw, various by-products, etc. Almost as high a proportion of the starch equivalent was supplied by temporary and permanent grass: 12·5 million tons out of 21·2 million tons. Our present problem is to see how much protein and starch equivalent we can continue to produce, because this affects the supply of first-class protein available for human beings. This is the justification for the emphasis now being laid on grassland improvement; we are compelled to plough some of our present grass in order to increase our acreage of cereals and potatoes, and so the remaining grass must be improved to make the smaller area as far as possible do the work of the larger one. Fortunately the methods are well known, and they have been so well described in this Journal that there is no need to repeat them. We can, too, take a leaf out of the German farmer's book and make more use of silage as a means of saving some of the excess of summer grass and holding it for the winter.

Increasing our area of cultivated land.—Although we cannot, like the Germans, requisition our neighbours' lands and crops, we possess a considerable reserve of land that can be converted to better use than was economically practicable in peace-time, and its improvement has become an urgent war-time necessity. Much of this land has been cultivated in olden days, especially in the great wars of Napoleon's time and in 1914-18, and methods of utilisation are now known. Some Agricultural Committees are already doing a great deal in this direction: Norfolk, for example, is starting on a 10,000 acre tract near Feltwell and some 4,000 acres in the Brekland region; when the full story of what is being done in other counties can be told the total will probably be impressive. It is now much easier than ever before to tackle this job; suitable tractors and other implements have been designed,

and numerous experiments have already been made to test various possibilities. The situation in regard to animal food supply is so serious as to justify every possible effort to improve it, and any farmer who, without prejudice to his output of grain and potatoes, can squeeze out from his farm a few more animals for the butcher, or more gallons of milk, or more eggs, and put them on the market, is doing a public service.

The curtailment of the grassland and of supplies of imported feeding-stuffs must of course reduce the numbers of livestock in the country, but the reduction need not be too drastic. On our own farm of 375 acres our head of livestock in the last year of peace was 112 cattle and a breeding flock of 300 Border Leicester ewes: in the summer our livestock population would be about 136 cattle and calves, and 830 sheep and lambs, this being well above the average.¹ We had 216 acres permanent grass and 133 acres arable, and we purchased some 85 tons of feeding-stuffs. We have now ploughed up 54½ acres of our permanent grass, thus reducing its area by 25 per cent., and our purchases of feeding-stuffs will this year be cut down 50 per cent. But the head of stock is not being proportionately reduced: the sheep are down only by 25 per cent. to 606 (225 breeding ewes and 379 lambs) and the cattle by 33 per cent. to 84. Even these reductions are greater than they need be on an ordinary farm where the density of stocking would be much lower. Our pig population is actually going up, but this is for a special reason: when we bought the estate in 1934 it comprised 75 acres of woodland, of which about 55 were felled by the vendor; we did not wish to replant so much, and so we are changing about 40 acres into grassland. The tree roots effectively prevent ploughing up, so we turned in some Tamworths, and they are such hardy, industrious diggers that they clear the ground, leaving us with only a few shrub-like growths to tear out with a tractor, after which seeding by hand becomes possible.

Scotland has a particularly interesting set of problems in connection with the 10 million acres of hill grazings and deer forests lying below the 1500 ft. level, some of which at any rate offer hope of improvement by the introduction of mixed cattle and sheep grazing, by cutting bracken, by drainage, liming, manuring, etc.

However, it is no use improving the remaining grassland unless this is accompanied by a drastic culling of livestock that are not pulling their weight on the farm. Milk recording has helped a great deal here: in England and Wales the Societies show a steady rise in yield from 473 gallons per head in 1919-20 to 546 gallons

¹ The average for England and Wales for 1930 was, per 100 acres farmed land, 27 cattle: 72 sheep; our figures were 36 cattle: 263 sheep.

per head in 1935-36.¹ Many instances could be given of benefits derived from culling, and the present inducements to sell should encourage this.

The ploughing up of grassland in England will, it is hoped, encourage the folding of sheep on arable land. This is one of the surest ways of maintaining fertility of the light soils, especially now that the supply of farmyard manure is likely to be restricted owing to the curtailment of purchased feeding-stuffs. If the war goes on long enough we may yet see some of the old folding breeds become more popular. But, in the meantime, the Border Leicester-Cheviot remains high in favour, and the more culling we do in the south the more we shall have to go north to replenish our flocks. So we naturally hope that good breeding stocks will be well looked after.

III. THE PROTECTIVE FOODS.

Vitamins and Minerals.—These will undoubtedly play an increasingly important part in the war; they include vegetables and fruit in addition to milk and potatoes already mentioned. Our consumption of fruit and vegetables had been steadily increasing; it is difficult to estimate the quantities eaten because of the large amounts grown in private gardens and allotments. The last peace-time figures indicated a consumption of the order of 115 lb. of fruit and 100 lb. of vegetables per head per annum. Of the fruit a large part was imported; the most popular were apples, oranges and bananas. These imports are now very heavily curtailed, and we are down to the supplies produced at home. This makes a severe cut into the important protective foods and makes it imperative that those who can grow fruit should intensify their production.

Black-currants come first in the list of fruits as suppliers of vitamins and minerals, but strawberries and raspberries stand high also. On the other hand, some of the luxury fruits, grapes, pears, melons, etc., are much lower down, and so we can console ourselves if we have to go without them.

Vegetables were always mainly produced at home, and their cultivation was far more widely spread than that of fruit. They serve three purposes: as nutrients, as sources of vitamins and minerals, and as flavouring materials. Their nutrient value is not high, though carrots, broad beans, and peas are fairly good; and the protein of the green leaf vegetables, spinach, Brussels sprouts, etc., though small in amount, is good biologically. The

¹It is not necessary to remind readers of the difficulty of defining "annual milk yield" and of the resulting uncertainty of many of the statistics. The Milk Recording Societies have adopted a precise definition and stuck to it, so that their figures are really comparable.

chief value of vegetables lies in their protective and flavouring properties. Fortunately, vegetables are as good as fruit for supplying vitamins and minerals, and so long as we are well provided with them our dietary need not suffer. But economy of transport is even more important than for potatoes, as vegetables rapidly lose some of their vitamins if they are kept long. Local self-sufficiency must be the aim, and farmers having access to large town and city markets should enquire into the possibilities of vegetable production. Great efforts are being made to increase the output from allotments and gardens, but these will not nearly suffice, and there will be a big demand for more. Sprouting broccoli and Brussels sprouts are among the best vegetables for supplying vitamins and minerals, while some of the luxury vegetables, asparagus, etc., come near the bottom of the list. Onions are low in vitamin content but have considerable value for flavouring; many people never appreciated them till they became scarce in 1941, and one can expect a sustained demand in 1942. With the curtailment of meat, and the greater predominance of bread and potatoes, the need for more vegetables will be emphasised, and, incidentally, those who have charge of the feeding of land girls and of other workers new to the land should take special care that their dietary includes abundance of fresh vegetables so as to keep them in full health and efficiency.

An Eye on the Future.

It is one of the special characteristics of good farming in Great Britain that it is never done for the day alone; an eye is always kept on the future. It is an old saying that a man should live as though he were going to die to-morrow, but farm as though he were going to live for ever. Many people are a good deal worried about the effects of the present drive for intensive production by using more artificial fertilisers, especially sulphate of ammonia. There would be no fear if abundance of farmyard manure were available, but, unfortunately, this will not be the case, and restrictions on feeding-stuffs will cut supplies down. This is particularly sad because more straw than usual will be available, and the demand and prices for the finished product, whether meat or milk, are sufficiently good to justify every effort at improved production.

The chief dangers of the present position are that the soil may become acid, and that it may lose organic matter or "humus." In spite of the activities of those responsible for the Land Fertility Scheme there remains a great area of land in need of lime. Potatoes, oats, alsike clover and grass suffer least from this defect, but it is a defect, and the official adviser should be consulted as to whether or not lime is needed; the advice costs nothing and it

may save a great deal. The full value of fertilisers cannot be obtained on acid soils.

The farmyard manure should go on the potato or the root crops, but there is not likely to be enough to prevent some of the arable land losing organic matter; this, however, rights itself when the land is put back into a grass-clover mixture, as it should be after some three or four years of arable cropping, and organic matter again accumulates.

The really important thing is that strong efforts should always be made to improve the farm all through the war years. There is a lot of public support now for agriculture. Take full advantage of it. Use every bit of help you can get from whatever quarter for bringing more of the marginal land into cultivation, for getting rid of poor pasture by ploughing it up, and after cropping lay down a better mixture; drain and lime land that needs it, and get the hedges in order.

It is inevitable that there should be more planning in the post-war agriculture than in the past. When peace comes the problems of reconstruction will be as grave as those now confronting us, and will be rendered more difficult by the sharp divisions of opinion which in war-time are in abeyance, but are likely to arise again. Agriculture must obviously put its own house in order, and in doing this it would be very helpful if the numerous surveys made before and during the war could be systematically worked up into County Reports, as was done by the first Board of Agriculture at the end of the 18th and the beginning of the 19th centuries. This would enable the agricultural position to be set out in proper perspective. But agriculture will also have social services to perform; it will be called upon for help in reconditioning suffering people and reconstructing our national life. Some experience was gained after the last war, and this should prove useful when the time comes. Finally, agriculture will always have to play its part in national defence, for we cannot assume that this is our last great struggle; science and engineering will continue to furnish new resources which in the hands of adventurers may be used for conquest and plunder, and national security will always have to be a paramount consideration for peace-loving people. Our agriculture must be developed with the double purpose of serving peace-time needs and of assuring a war-time dietary when next the need arises. We need not, like the Germans, live permanently on a war-time dietary, but we must be able to produce it at very short notice.

For these various reasons our agriculture cannot be left to drift. We are not likely to attempt restrictive measures like some of those adopted by the Germans. For us there is a much better way. We know how much food the nation needs, and when

peace comes and international trade starts up again we can decide what is the minimum below which home production should not fall, and allocate our remaining requirements by trade agreements with the various parts of the Empire, the United States, and other countries. The home production could be allocated on a county basis by the War Agricultural Committees, who could organise a Farmers' Reserve, similar to the Army and Navy Reserves, of men who undertake to deliver specified quantities of the various commodities to the buying organisation. The machinery exists in the Marketing Boards, and the methods would be the placing of contracts, as is done for milk and sugar beet, or the fixing of prices, as is now done for wheat, potatoes, livestock and other products. We are getting used to these methods now and learning how to work them smoothly. We should regard them as part of the permanent machinery of the future and improve them accordingly. We cannot hope, even if we wished, to get back to the old days of unrestricted competition between home and overseas producers; our great problem will be to combine the necessary degree of planning with that freedom of action that has hitherto been characteristic of British agriculture, and to which so much of its high technical efficiency is due.

RECONSTRUCTION AND DEVELOPMENT IN EASTERN POLAND, 1930-39

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Meeting of the Society, 3 November 1941

THE years of my title have been chosen as the first and last of a series of visits to Poland. I write of the country as I saw it in those years and often use the present tense where the past would now unhappily be more appropriate. 'The statistics throughout the paper are from the 'Concise statistical year book of Poland, 1937.' Except where otherwise stated, population data are for 1931 and crop data for averages of 1932-36.

It has been Poland's tragedy to have no natural boundary either to the east or to the west but only to the north and the south; in consequence during its long and eventful history there have been expansions and contractions eastwards and westwards like the movements of a concertina. Certain boundaries became definite after 1923, and had Poland been left in peace these could have become quite effective. The eastern regions fall into that remarkable strip of Europe, lying between longitudes 22° and 28° E., only as wide as from London to Holyhead, into which the surging movements of peoples from east and from west have compacted more nations than in any equal belt in the world; seventeen principal races and a host of minor ones are found along a stretch no longer than the return journey from London to the Shetlands.

Of the several nationalities in these eastern regions of Poland the most important are the Ruthenians, the Jews, the Germans, and the Lithuanians. The Ruthenians inhabit a belt of country running north and south along the Polish border and they are divided between Poland, Russia, Czechoslovakia, and Romania. There are several groups which have little if anything in common: the White Ruthenians of the north; the Polesians; the Red Ruthenians of Wołyń and Galicia; the Huculs of the Carpathians. In the main the White Ruthenians are Greek Orthodox while the Poles are Roman Catholics; their upper classes always freely intermarried with the Poles but the peasants remained more distinct. The Red Ruthenians are Greek Catholics. In recent years a political division has appeared chiefly among them: the Ukrainians, who are aiming at an independent Ruthenian nation. The idea of independence for the Ukraine was severely repressed in Russia, but the Poles admit the right of free national development and fostered Ruthenian schools and co-operative societies. Political troubles arose in the south between 1920 and 1935, when there were political assassinations, but I heard nothing of them in the northern regions. It is absolutely impossible to draw any frontier on purely ethnographical lines; indeed until recently the peasants did not classify themselves on national lines, but by their religions. The population is of similar structure throughout: in the towns Poles and Jews preponderate; in the country, Poles and one of the Ruthenian groups, but very few Jews. In Tsarist times this had been the region of large estates; the landowners were mainly Poles and the Ruthenians were mainly small farmers

and peasants. Under the Polish Government these large estates were being broken up, but some, like the Radziwiłł estate, chiefly forests, survived.

Geographically these eastern regions fall into four well-defined areas: the high ground (300-500 m.) of Podole in the south; the lower ground (100-200 m.) of Wołyń farther north; the still lower ground (below 100 m.) of Polesie in the centre; and beyond that the higher ground of the Nowogródek and Wilno regions.

Polesie

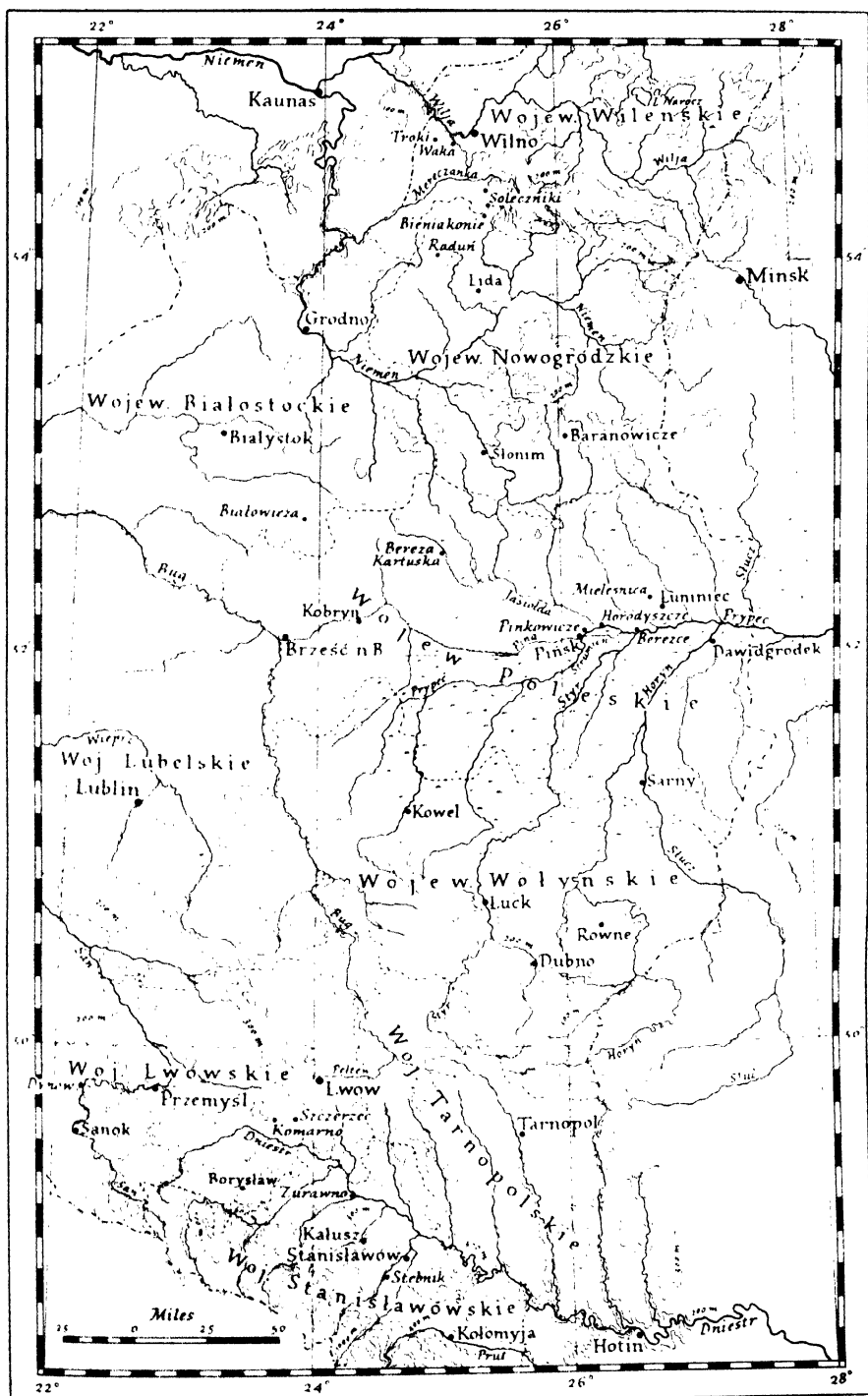
Polesie comprises the Pripet marshes, a huge oval basin lying east and west along the centre of which runs the river Prypeć. Only part is in Poland; the rest stretches into Russia as far as the Dnieper into which the Prypeć empties; a southern extension goes almost to Kiev nearly 400 miles down stream. The Polish part is only about 100 metres above sea-level, and the river has still 900 miles to flow before it reaches the sea, so that the fall is extremely slight. The rainfall is low, averaging at Sarny 560 mm., and the summers are hotter and drier, and the winters cooler and more severe, than is usual in Poland.

The most numerous and important of the tributaries of the Prypeć come in from the south and would naturally have gone to the Baltic. But in Polesie their way was blocked by the ice and dunes of the glacial period, and so they formed the Prypeć and found a way out eastwards. The watersheds that divide the Prypeć system from the Bug and the Niemen are very low and ill defined, so that these river systems trespass a good deal on the Prypeć, and the trespassing varies with the seasons and the human activities. The very slow flow leads to short circuiting between one branch of the river and another: the anastomosis of the physiographers. Flood time is March and April, and low water is in September and October. Stanisław Pawłowski gives an admirable description of the geography of the marsh region in *Congrès Internat. de Géographie, Varsovie, 1934: Excursion Polésie et Białowieża*.

The marsh forms a vast plain at three slightly different levels. Along the river are the mud flats, chiefly mineral matter brought by the river; just beyond and slightly higher is the peat which forms a layer 3-6 m. thick; this forms by far the largest part of the marsh, and as the water is well charged with mineral water and the substratum is often chalk the vegetation has been of the mineral peat type, giving rise to "low moor" peat like that of our fens, and well suited to cultivation when drained. At a higher level is the acid peat more suitable for fuel and industrial use.

The plain is broken by sand dunes which are older than the peat, and play a very important part in the life of the country as they are the only parts not waterlogged and liable to flooding. Dunes are more extensive here than anywhere else in Poland or indeed in Europe. They are up to 20 m. in height, and form ramparts which may be as much as 5 km. long; the east slope is scarped, the west is gentle. They are or were covered by trees; after these are removed the soil can be cultivated for a time but it then loses its humus and becomes a blowing sand.

About one-third of the province is forest. In the northern part pine and fir predominate; in the south there are more deciduous trees: oak, ash, and



alder. The fauna is varied; it includes wolves, bears, deer, and even elk; by the water there are beavers. It is the forest that gives the name to the region: Polissya, as the natives call it, in Polish Polesie, means "the country along the forest" (*las*). The name has nothing to do with Poland, which probably means the plain dweller, from Pole, plain, or in modern usage, a cultivated field. I am indebted to Professor E. H. Minns for information on these points.

The lakes are very characteristic features; there are more than four hundred having an area larger than 1 km²., although they have little economic importance.

Polesie has been inhabited since very early times by a group of what are now the Ruthenians, but the special environment and the isolation have given them an entirely distinctive character and they recognize no affinities with any other people. They call themselves Polesuni; the Poles call them Poleszuki. They belong to the Greek Orthodox Church, and they average 70% of the rural population; the Poles, including the estate owners, average about 12% and in some districts fall below 10%; they are Catholic. But the population is sparse; Polesie occupies some 10% of the total area of Poland, but it had only 1.13 million people in 1931, 3% of the total population.

The towns have only 13% of the population and it is much more mixed than in the country; about half are Jews, this being a higher proportion than in any other province except Wołyń; 30% are Poles, the rest are Polesians with a sprinkling of Ruthenians and Russians. The largest town is Brześć, on the Bug (Brest Litovsk), the administrative centre; it had a population of 50,700 in 1931, but was rapidly growing.

The centre of life and culture for the marshes is Pińsk (32,000 in 1931), built on a spur called Zakorodzia, where the ways from Wołyń, the Vistula, and the Niemen all meet. It is an ancient city dominated by the seventeenth-century cathedral and monastery associated with the Jesuit missionary Andrzej Bobola, who was put to death by Cossacks and whose relics, after many vicissitudes, were returned to Poland with much ceremony in 1938. It is claimed that a synagogue has been here since the ninth century. As they were built mainly of wood few old buildings have survived, nothing comparable in interest with the towns in or near the Vistula such as Toruń or Chełmo. The main street is cobbled, the shops and houses small, but the large market-place is very interesting on market days, when it is thronged by peasants bringing in their wares: farm produce, hay, and some pottery.

Pińsk is an important collecting and distributing centre: the region exports timber and timber products, meat, fish, geese, mushrooms, and many minor foods, and it imports wheat, rye, and tobacco, mainly from the central and western regions. There are a match factory, timber yards, and two wood factories: it is claimed that plywood was invented here. As usual in Poland the commerce was largely in the hands of the Jews, who never seem to miss an opportunity.

There are no good roads and travel is mostly by water or on foot along paths that often follow the tops of the sand-dunes, or on cart-tracks running along the base where the moisture binds the sand and makes it firmer. A



Market place and Dominican Convent church, Łwów



Wilno: The church of Ostra Brama



Ruthenian cottage, Dniestr valley



Cottage of old style, Berezce, Piiisk marshes

motor car is of little service as the roads, mostly earth tracks, become impassable with rain. The pleasantest way of seeing the marshes is to charter a little steamer at Pińsk. The journey starts on the river Strumień, then on the Pina and then the Prypeć, affectionately called the *Starucha* (the old lady) because it has several times changed its course but always comes back to the old one again. There is much bird life: storks, herons, grouse, and many lesser birds; one sees geese, cattle, and horses, and in August many heaps of hay piled up and around tree stumps. The peasants travel in long narrow rowing boats saved only by skilful manipulation from being capsized by the wash from the small steamer. Fishermen are busy with large nets fixed on frames and live in strange little huts, called *Kurin*, made of reeds.

Villages are scarce and far apart, usually compact, the cottages are of wood and one storey only; most are plastered with loam and whitened, though some of the older ones in the centre and north are of rough timber unwhitened and with small windows; very occasionally one is of brick, built by an emigrant returned from the United States with money in his pocket. One meets these people all over Poland: they never lost the desire to come back to their native village, though their enthusiasm is not always shared by those children who remember life in the States. In general the cottages have three rooms, in one of which is the stove, made of brick; they have wooden floors, broad seats round the walls or beds made of hay sewn up in coarse material. Commonly some sacred pictures hang in the corner, but with no lamp. The houses are roofed with thatch, sometimes fastened down with boughs of trees. The doors in the buildings are of tree trunks with their boughs still attached to save mortise and tenons.

The houses are of course very combustible and once a fire starts much of the village may be burnt. Till recently some witchcraft survived, certain old women knew the incantations to be spoken as flowers are thrown into the fire to bring it under control. Deliberate firing of haystacks for motives of revenge sometimes occurs, and the stacks of the villagers are placed close together on a raised stretch of sand so that if one peasant burns another's stack his own will be lost in the conflagration. To burn a man's house and plough up the site is the traditional peasant vengeance, while the firing of the landowners' stacks or barns was in the past one of the first signs of dissatisfaction or political unrest.

The cottage gardens produce vegetables, some of which however are lost by flood, and most peasants have beehives made out of large hollow tree trunks, with a few holes bored for the bees to get in and out, and a door for the removal of honey. The region has always been famous for its honey.

The Tsarist government had kept the whole marsh region in an extremely backward state for strategic reasons. Few developments or drainage works were undertaken, and no roads were made. There were no schools and the people were almost all illiterate. The isolation was almost unbelievable. During the years 1914-18 some of the peasants did not know that there was a war on, and only suspected something because no official came to collect the taxes. In some parts a motor car was till recently such an object of terror that the peasants crossed themselves and prayed when one passed them. In the villages we were introduced as *Americani*. I endeavoured to explain the

difference but was politely informed that the villagers had never heard of England and that anyway all foreigners were Americani.

Everything had to be produced at home. Utensils, implements, even locks for the doors were of wood; indeed Pawłowski speaks of these people as living in the age of wood. Yet they have distinctive peasant arts: their weaving in particular is very attractive. The material is linen. The flax is grown and retted locally, the fibre is spun and dyed with vegetable dyes, also home-made; a red colour is made from alder, a brick red from wild thyme, copper sulphate added to these (a recent device) gives a blue, while peat extract gives a dull black. The thread is mounted on the loom as the warp, but in the best of the work the weft, instead of being carried in a shuttle, is on a needle which is skilfully threaded across. Very attractive patterns thus become possible, but of course the work is slow. The most beautiful work comes from the wettest and most inaccessible villages, as here the women have most time on their hands and least material. Bast footwear and sheepskin coats are common, and the women wear brightly printed cotton squares on their heads.

As usual among illiterate people the age of marriage is low, even for Poland, no fewer than 25% of the brides being under nineteen, while the birth-rate is the highest in Poland (34 per 1000), as is also the natural increase in population (19 per 1000). This has necessitated a good deal of emigration to the western regions during spring and summer, but the peasants return for the winter. Formerly there was much migration to Brazil and to North America.

Another consequence of these conditions was a tendency to idleness and to drink, and unfortunately it was vodka. Men and women both indulged, those who had the money sometimes rather heavily. Richer farmers would sometimes buy one hundred bottles of vodka for a wedding feast: without it there would be no festivity. Fortunately more temperate habits are developing in consequence of the financial crisis, education, and it is said, the spreading Baptist movement.

A survey of Kobryń (75 miles west of Pińsk) recently made by Jakubowski¹ gives a concrete illustration of the conditions. The population of this district increased by 58.6% during the ten years 1921-31, when it stood at 114,171; much of this was by repatriation. Eighty-five per cent were dependent on agriculture and only 6% on industry and crafts; subdivision of the land therefore necessarily continued and over 50% of the farms were under 5 hectares in size. The dietary, as elsewhere in Polesie, is: Rye bread, potatoes, cabbage soup (*barszcz*), millet porridge, and in the marsh fish, especially the celebrated *Wnuj*. The only fats available are lard and linseed oil and then only during periods of hard work. Sugar is a great rarity, eaten only by the richer peasants during festivities, which fortunately for them are fairly common. As occasional treats are white bread, rolls, and herrings.

Improvements effected by the Poles.—The Poles set about improvement in

¹ J. Jakubowski, 'Stan Społeczno-Kulturalny Wsi Pow. Kobryńskiego,' *Zagad. Pracy Kult.*, Rocznik II, Warsaw 1936. I am indebted to Dr. A. Waligórski for this reference and summary and for other help in preparing this paper; also to Professor Zółtowski of the Polish Research Centre, to Dr. and Mrs. Kleczowski, and to Mrs. Conbridge-Pataniowska.

two ways: education, and better economic conditions. Education was made compulsory between the ages of seven and thirteen, but subject to the proviso that no child is expected to go more than 3 km. to school. It was necessary to start from the very beginning, and as there was very little money the schools were still inadequate right up to the war. One I visited in Bereźce opened in 1928 and had in 1934 one hundred and seventy-four children but only two teachers, and two rooms with two classes in each, the walls bare and undecorated, the seats and desks uncomfortable. But the teachers greatly impressed me.

The improvement in economic conditions required drastic changes in the land system, drainage and other land amelioration, and better marketing methods. Throughout these eastern regions the peasants' holdings were in strips scattered so that each should have his share of good and of bad land. The crops were arranged roughly in three courses: rye (the most important), other grains, uncropped fallow. The chief crops and their areas in thousand ha. were rye (278), potatoes (134), oats (84), barley (36), wheat (24), linseed (14), and millet (11). But the rest was no longer all uncropped. One of the great improvements fostered by the Poles had been the increased growth of potatoes and other crops in place of the old fallow. But the yields were still low, only about one-half those obtained in the west. Cattle (Polish Red) were increasing and were in 1936 more numerous per thousand of population than elsewhere in Poland: they were used for meat rather than milk. Sheep are also numerous here and to the north; elsewhere in Poland there are few; they provide wool for weaving and their skins are made into coats.

The land was not economically used. One holding of 20 hectares had 2 ha. of rye, 2 of oats, 1 of potatoes, and 4 of meadow, and 10 in wild grass and other use. On the large estates also there was much wild land: a 16,000 ha. estate had only 110 arable, cultivated, however, on a good rotation and yielding well: potatoes, oats, red clover, rye, white clover, 200 ha. were forest, and the rest was wild grass; it was devoted to the breeding of horses and of the Red Polish cattle. Even in 1938 the estate had not fully recovered from the destruction wrought by the Germans in 1914-18. But improvement was going on: fifty-five thousand holdings, an aggregate of 500,000 ha., were consolidated in Polesie between 1919 and 1936.

A great scheme for the development of Polesie was started in 1931. An annual income of 300,000 zł. was provided of which 10% came from local taxes and the rest from the Central Government, the region being as yet too poor to pay more. An experimental station had already been started in 1925 at Sarny on the river Stucz to study the possibilities of agricultural development. First the land had to be drained. Main channels may be up to 12 m. wide and 4 m. deep, the secondaries, 1½ m. wide, 1½ m. deep, spaced at 25-100 m. Then the land is ploughed and the native vegetation completely buried, fertilizer is added, and hardy grasses sown: *Dactylis*, *Alopecurus pratense* and *beckmanii*, *Festuca rubra* and *pratensis*, *Phleum pratense*, *Poa pratense*, *palustris*, and *serotina*, also Swedish and red clovers. The total cost may be 220-350 zł. per ha.: 100-150 zł. for main drainage, 60 zł. for the trenches, 60 zł. for seeding, and 60 zł. for manuring. The Government paid 6-12 zł. per quintal for the hay, so that the cost of reclaiming was about the price of

50 q. of hay. The cost is borne by the landowners or by a State loan obtainable at 3%. Striking results are obtained. The wild vegetation, rushes and sedges, myosotis, spirea, potentilla, herb robin, mint, *Polygonum aviculare*, buttercup, but very little grass and no clover, may yield 10 q./ha.; improved land yields six or eight times as much and of better quality. A peasant with 10 ha. had before improvement three cows only, but could not feed them properly; after improvement he had eleven cows much better fed and had hay to sell. This kind of improvement could be widely extended if Russia would consent to a general lowering of the river levels.

The manurial results are much like those obtained on the fens of Holland, showing how marsh conditions smooth out the effect of climate. The same curious 'reclamation disease' occurs, requiring the addition of about 35-55 lb. copper sulphate per acre. The possibilities of improving the arable crops are also considerable; while the average yields in the marsh for potatoes are about 80-90 q./ha., those at Sarny are 300-400, and 20-30 of rye against an average of 8-10 q./ha. In the Pińsk powiat some 10% of the 130,000 ha. of wild land had been reclaimed between 1935 and 1938, while in the whole province 52,000 ha. has been reclaimed and no less than 200,000 ha. has been cut out of large estates, to make small farms.

Considerable progress was made with horse breeding, the best of which was done on the larger farms. The Army was the chief buyer and set the standards, but Polish country people are very fond of horses, and landowners and peasants are equally proud of a good turnout on Sundays and feast days. Some of the children, girls as well as boys, are excellent riders. The native marsh cattle were not so good as the Polish Red, and were therefore being displaced by these: the native pigs had some useful qualities, but were less useful than the Large White crosses.

Proper exploitation of the timber was organized. It was railed to Gdynia, or floated along the canal to the Bug and thence to the Vistula, or worked up at one of the local factories. The fishing was also being improved. A biological research station was established at Pińsk in 1937 to study the biology of the rivers, beginning with the distribution of the crayfish and its parasites, and the bacterial disease, which twenty years ago had almost exterminated them. They were a valuable export, mostly to France. Peasant industries were encouraged. Cooperative societies and Peasant Women's Associations were formed, and attractive shops established in the chief towns so as to avoid the bane of peasant arts and crafts, the middlemen who underpay the peasants and overcharge the purchasers. The culture of apples and of plums was also developing, especially in the west on the loamy soils. The wild plants were also being better used: medicinal herbs, berries such as cranberries (*Oxycoccus*), reeds for thatching and for transport to Warsaw to be made into mats. An annual exhibition, the Jarmark Poleski, was to be held each year in Pińsk in August to show the progress and the products.

The northern regions: Nowogródek and Wilno

North of the marsh is the województwo (province) of Nowogródek, well above the marsh level; it is traversed by the Niemen and by the railway from Warsaw to Moscow: the country is undulating, about a quarter is forest, and

about 35% is cropped, chiefly with rye, oats, and potatoes. The yields are higher than in Polesie or in Wilno, much more barley is produced, and the peasant's dietary is better; barley porridge takes the place of the millet porridge of the marsh and the buckwheat of the north. The houses are of wood logs or planks roofed with straw, tiles, or sheet iron; many of the fields are large.

The population is almost entirely rural, and less than 10% live in the towns. As throughout the eastern provinces the population differs greatly between town and country; in the town about 40% are Jewish, 45% Polish, and only 11% White Ruthenian, while in the country less than 4% are Jewish, on the average 53% are Polish, and 42% White Ruthenian, but in some districts more. The largest town, Baranowicze, had only 23,000 inhabitants in 1931; it is the railway junction where the Warsaw-Moscow line crosses the north-south line from Wilno to Lwów. It has no architectural or historic interest, yet contrived to make itself look very gay for the soldiers' fête day on August 15 to commemorate the Miracle of Warsaw, the defeat of the invaders of 1920. Only two other towns have more than ten thousand inhabitants; Lida and Stonim.

The province of Wilno is more picturesque and historically more important. It is in the moraine country, its lakes including Narocz, the largest in Poland; its many hills are low but steep, usually well wooded; the plains are wooded and much cultivated. The cottages are of wood, with straw thatch. The soil is sand or clay, usually poor and acid, and less productive than that to the south; the chief crops are rye, oats, potatoes, but little wheat or barley. Buckwheat however is grown more extensively than anywhere in Poland, also flax and peas. There are far fewer cattle than in Polesie or Wołyń, and fewer per 1000 ha. than in Nowogródek; on the other hand there are many more sheep than elsewhere in Poland and they were increasing, the figure for 1937 being four times that for 1921.

As in the other regions much of the land is in small holdings, about half being 5 ha. or less (*i.e.* below the poverty-line), and 15% were actually below 2 ha. There is much strip farming, some strips being very small. The population is not quite as largely rural as in Nowogródek or Polesie, some 20% being urban in 1931; in the country 60% are Polish, 28% White Ruthenian, and 3% Jewish, while in the towns 63% are Polish, 29% Jewish, and 3% Ruthenian. Almost all of the Ruthenians were small farmers, wanting only to be left in peace, free to use their own language and practise their own religion; they are mostly Greek Orthodox now, though in the old days they had been Greek Catholics. White Ruthenian secondary schools had been started; I was informed that they were not a success through lack of pupils. As elsewhere in the eastern provinces illiteracy has been bad; 60% of the rural population above ten years of age were unable to read and write in 1921; this was reduced to 33% by 1931.

I have visited several well-managed large estates. One south of Wilno had 3000 ha., 1000 of which was arable; its yields of rye and potatoes were double the average, though even here oats and barley did not do well and wheat was not attempted. The grassland was being improved as on other good estates; and a good herd of Frisian cows gave high and increasing

output of milk; cheese was successfully made. The pigs were Large Whites, a popular breed in Poland, suggesting one of the many ways in which we shall be able to help after the war: the large building housing them was provided with loopholes out of which the guards could shoot the wolves in winter.

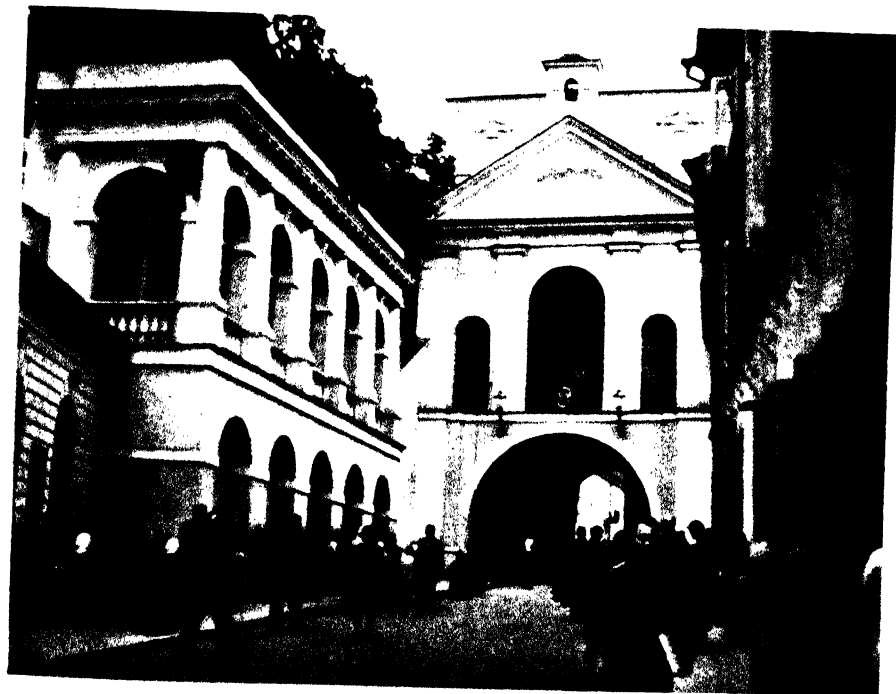
On another estate, equally well run, special efforts had been made to develop the fishponds, a great industry in Poland. Carp are the most usual; they are fed with lupin seeds, and thousands of acres of water are devoted to them; indeed on some estates I was told that more could be got out of a hectare of water than out of a hectare of land. In 1938 the annual production of carp in Poland was about 12,000 tons, and another 14,000 of carp and pike came from the lakes, 26,000 tons in all, nearly sufficient for national needs. Most of the carp was said to be eaten by the Jews as a "meritorious act." One estate producing trout had ingenious devices for attracting insects to the water; electric lamps were submerged just below the surface and lighted at night; horse flesh was also given. A special society is concerned with these pond fisheries.

It is impossible to speak of these country houses without a feeling of regret that they should have suffered so badly and for so long. The breaking up of the big estates is bound to affect them. Hospitality is an ancient tradition with them; one hostess told me that in her grandmother's time rarely less than fifty had sat down to dinner, and in her own childhood they were rarely less than fifteen, including the French and Polish governess and the poor relations. As in England the country houses expanded in the 1860's, when money was plentiful though for different reasons than here; the emancipation of the serfs brought in compensation funds, timber began to have value, and the development of transport enabled agricultural produce to be sent to distant markets. A period of fluctuating fortune followed, ending with the war of 1914-18 when the Germans destroyed or plundered everything they fancied, including the books. Then came twenty years of slow but steady reconstruction to the high standard finally reached in 1939, and now, this renewed devastation.

Two central agricultural experimental stations deal with agricultural problems. At Bieniakonie, 35 km. south of Wilno, cereal crops are studied under Professor Łastowski. The central flax station, under Professor Jagmin, at Nowa Wilejka, near Wilno, deals with home-grown fibres; it is located here because this province is the largest producer of flax in Poland, and Poland is the second largest producer in the world. Its programme illustrates the ingenuity with which the Poles were attacking their problems. Raw textile materials were imported in considerable quantities and paid for by subsidized exports of cereals, sugar, and other agricultural produce really needed at home. The annual consumption of sugar per head in Poland was less than half that in England. Tales were current on the frontier of considerable smuggling back into Poland of sugar sold to an adjoining country at less than the home price. It was decided therefore to replace some of the cereals with fibre crops which in any case were worth about three times as much per hectare. Cotton and jute, the two most needed, cannot of course be grown in Poland, but investigations were started to see if flax, hemp, and abutilon



Market place and Dominican Convent church, Łwów



Wilno: The church of Ostra Brama



Strip cultivation, Wilno province



Country house in Dniestr valley



Professor Jagmin and experimental garden for fibre plants: Wilno

could be changed into something sufficiently like them to be effective substitutes.

The exploitation of the forests involved some difficult problems, so much damage having been done by the Germans. About 20% of the province is forest, nearly half being State-owned and the rest in private hands; but the State exercises considerable control, requiring a proper survey and a ten-year plan of cutting and regeneration to be approved by the Forestry Department and carried out. Much timber is exported as coniferous sawn wood, but efforts are made to sell it in manufactured form; the Wilno district produces some 70% of the plywood and veneer of Poland.

Transport however was difficult. The natural outlet is the Niemen, but unfortunately this passed also through Lithuania and Prussia, and the navigation restrictions made it useless. However the Poles never accept defeat, and they established several pleasant 'Plages' upstream from Wilno. Libava was the nearest port by rail, but it lacked all facilities; Memel was also near. But the only practicable ports were Danzig and Gdynia, a long way off, and Danzig was becoming politically hostile. Timber was Poland's chief export and we were the chief buyers.

The administrative and cultural centre of the province is Wilno, one of the most attractive cities in Poland. It is built on the moraine hills, and the Wilja, a tributary of the Niemen, cuts through it in a sinuous course. The hills are not high, but they are steep, and they effectively prevent any rectangular layout of the city; they afford however many pleasing views over the city. It was a favourite residence of King Sigismund Augustus in the latter part of the sixteenth century, Poland's golden age, and must have been magnificent if we can judge by the few surviving Gothic churches, and especially St. Ann's, and the remains of the Castle; these were being cleared and conserved. Here Barbara Radziwiłł lived and was buried; till this war her crown still rested on her coffin in the Cathedral; from here too started King Stefan Batory's road to Polotsk; it was reconstructed by Catherine the Great and planted with beech trees, and is still in use.

Like other Polish cities Wilno suffered greatly in the wars of the seventeenth century; some of the churches were rebuilt in the Baroque style with Italian help; in the early eighteenth century there was damage by fire, and the new churches were Rococo; later came the more severe classic styles; in the nineteenth century the Russians introduced some Byzantine work. From the hills one gets the impression of a city of spires and chestnut trees, and as one wanders down the narrow winding streets one finds many little courtyards often adorned with arcades and gardens and pleasing nooks and corners. Window boxes of flowers are popular; the police stations led the way. The people are attractive, with soft voices and a singing intonation. One can understand Piłsudski's affection for Wilno and his declaration that it was one of the most beautiful cities in the world; he was born here, and here his heart is buried in his mother's grave, which is placed among the soldiers' graves, as befits the mother of a warrior.

The University was founded by Stefan Batory in 1578 as a Jesuit Academy; it grew into a great seat of learning but was closed by the Russians after the 1830 insurrection and the Polish language was forbidden. Many stories still

survive of the repressions from then on to 1922, when it was reinstated by Piłsudski. But it is pleasanter to think of Napoleon's connection with Wilno; he passed through it in July 1812 on his way to Moscow, making a speech that sounds curiously modern about his noble aims and devastating intentions; but he came back in October in a sledge, travelling under another name. The road on which he passed was shown me and the story told of how he was nearly captured by Cossacks but was saved by some Poles who, my informant added, were thereby the cause of Waterloo. Caulaincourt mentions the Cossacks but not the alleged narrow escape.

Wilno is also an important religious centre. Some of the old Russian Orthodox still survive as the Eastern Orthodox Church of Ancient Rite; their predecessors had fled hither in the time of Peter the Great because they disapproved his ecclesiastical innovations. At Raduń is a well-known Rabbinical college, while at Troki are the Kariem, an interesting sect of Tartar origin who came from the Crimea in 1398; they combine many of the tenets of Judaism, Muhammadanism, and Christianity. But as only those born in the community can become members, and no converts can be accepted, their numbers are falling. They live very strict lives and are greatly respected.

The southern regions: Wołyń and Eastern Galicia

South of the marsh regions the country rises in two steps: first comes Wołyń, a plain some 200 m. above sea-level and about the same size as Polesie; then farther south on higher ground another two provinces, Tarnopol and Stanisławów, which correspond roughly to the old Eastern Galicia, together the same area as Wołyń, but with considerable differences, both natural and political. Wołyń, like Polesie, is a plain and has about the same rainfall, but it is more fertile; its chief crops are rye, wheat, oats, and potatoes; but with much more wheat and barley than farther north, yields are higher and cattle and pigs more numerous; there were however fewer sheep. Both at Dubno and at Kowel there were factories for preparing bacon, tinned ham, and lard for Great Britain and the United States. More land is in agricultural use (66% against 50% in Polesie) and only 20% is forest: it is altogether better country.

Eastern Galicia stands higher, has somewhat higher rainfall (690 mm. at Lwów), it has considerable stretches of fertile loess soil, and grows a much wider range of crops. Wheat is more important; indeed in the Tarnopol province it actually exceeds rye in area; there are also sugar beet, tobacco, flax, hemp, and two crops which are confined to the south-east and show the warmer character of its summer: maize and grapes for wine.

Both in Wołyń and in Eastern Galicia the rural population includes many Red Ruthenians, but with considerable differences. Wołyń was under Russia and, like Polesie, was kept in a backward state for strategic reasons; illiteracy was as common as in Polesie, no fewer than 85% of women and girls over ten, and 63% of the men and boys living in the country districts being unable to read and write in 1921. Eastern Galicia, on the other hand, was under Austrian rule, which was much more lenient than that of Russia or Germany. Some education was permitted, and in 1921 illiteracy was lower than in Wołyń, except in the remote districts where the people were very

backward. But as against this the Austrians tried to lighten their task of government by fomenting trouble between the Poles and the Ruthenians, and this left a host of difficulties for the Polish Government.

From its soil and climate Eastern Galicia ought to have been fairly prosperous, but it has always been poor. For centuries military adventures of many races have passed through it between Kiev and Lwów; the peasants have always been in the way, and have suffered accordingly. Dr. Styś (Vincent Styś, Arch. Towar. Nauk. Lwów, 1934) worked out the history of twenty of the villages and showed the devastation caused by wars, famine, and pestilence, especially cholera, smallpox, and typhus. Their high birth-rate had saved them during war and pestilence, but it nearly ruined them in peace time by putting too great a pressure on the land. The shrinkage in size of holdings is shown in the following figures taken from Dr. Styś' paper:

Annual change per cent in

	1787-1820	1820-50	1850-83	1883-1931
Population	+0.439	+0.610	+0.515	+0.822
Number of peasant holdings ..	+0.334	+0.903	+1.047	+1.098
Total area of holdings	+0.154	+0.116	+0.003	+0.349
Mean size of holdings	-0.163	-0.618	-0.778	-0.492

It has become a region of dwarf farms; in the various districts from half to two-thirds of the total holdings in 1921 were less than 5 ha., usually divided into strips. Approximately half the population is Polish and half Red Ruthenian, these being divided into Ruthenian and Ukrainian; in the south however there are fewer Poles.

When I first visited Eastern Galicia in 1930 there was still much war damage: broken bridges and wrecked houses. But the children had known only peace and isolation; they ran away terrified from our car. Women and girls in their nicely embroidered costumes, much brighter red than in the north, were working in the fields, cutting the corn with sickles; they had dimpled cheeks but not the high cheekbones of some of the other Slav groups. There is a certain amount of gloom and slowness of thought in their make-up. Maize enters largely into their dietary. The villages are compact; the houses of one storey only, made of wood frame only partly hewn, and filled in with straw coated with loam and then whitened or coloured pale blue. The straw-thatched roof is built in a characteristic overlapping style. Not infrequently there is no chimney; any smoke lingers indoors to help in the warming. Water is lifted from the wells either by the long pole lever or by a wheel.

Considerable improvement had been effected by 1936. The new cottages were of wood or brick, often with sheet-iron roofs painted red or green. Many thousand farms had been consolidated and some 200,000 ha. had been taken from the large estates and used for peasant holdings. Advancement became possible: one peasant in the Dniestr valley told me he had started with 2 ha., and by dint of hard work had now got ten. But the holding was not stable and his two sons would share it when he died. Fragmentation continues. New farms are exempt but a so-called "hidden subdivision" was going on, two or more families living on the same holding.

Some of the large estates were very productive however. One of 10,000 ha.

in the Dniestr valley region had 4000 ha. of forest, 2000 ha. wild grass, and 2000 ha. cultivated, of which 1200 ha. were arable, 200 pasture, and 400 fish ponds. The permanent staff numbered 120, summer workers another 200, with a further 50-200 at harvest. The wages were paid mostly in kind; they were, per annum in 1936: 15·3 q. (27 cwts.) of cereal grain per family; the use of $\frac{1}{3}$ ha. of land for potatoes; manure, horses, and implements being provided by the estate, but not seed; $\frac{1}{3}$ ha. of pasturage for the cow; free wood and medical attendance. Minimum wages were fixed by a commission. The number of families employed would probably not much exceed one hundred and fifty. Had it all been divided into 20 ha. holdings it might have provided for about two hundred families. But the same high standard of production could hardly have been maintained: the rich, clean milk, tuberculin tested, the high yields of grain, sugar beet, and colza. The house, though smaller than some, was a choice specimen of Polish taste and culture; the hostess, speaking English perfectly and with a considerable knowledge of Polish art, had a beautiful collection of books and prints, much attractive furniture and tapestry; it was a house where one could stay indefinitely. She was keenly interested in the estate and in the peasants; she ran a kindergarten, and medical services for the children; not wanting to make money out of the enterprise but simply to keep it going and help her people. It was a little island of the best Polish culture in a Ruthenian population.

The centre of commerce and of culture for this southern region is Lwów, called Lemberg during the Austrian occupation. It is an ancient city founded in 1250 by the Ruthenian Duke Lew, but Polish since 1340. Traffic was mostly by river; and the city was built on the Peltew, a tributary of the Bug, at the dominating point where a short portage linked up the Dniestr and the Bug over the low watershed; it was thus at the intersection of the routes from the Black Sea to the Baltic and from each of these seas to eastern and central Europe. It rapidly became a great trading centre and attracted settlers of many nations, including Scotsmen. It also achieved much political importance as the final bastion stopping the progress of invaders from the east: only once did it fall into foreign hands, and that was when the Swedes took it in 1704.

Lwów has the unique distinction of being the seat of three archbishoprics: Roman Catholic, Greek Catholic, and Armenian. It had become the chief centre of the oil and potash industries, and had important timber and other industries as well as much distributive commerce. Further, it became a great centre for higher education with a large university, technical colleges, and other teaching institutions. By 1931 it was the third largest city in Poland with a population of 312,000, about half of whom were Poles, a third were Jews, and the rest Ruthenians and others.

It has some beautiful churches and other interesting buildings, and on market days its big market square becomes filled with peasants dressed in gay colours, with their little stalls of fruit, vegetables, and chickens.

Summary of changes and developments

We can now sum up the changes in the eastern provinces since Poland took them over. The population was very mixed. In the towns the Poles

and Jews preponderated; in the country districts there were very few Jews; the Poles and one of the groups preponderated, Red Ruthenians in the south, White Ruthenians in the north, and Polesians in the centre. The Poles were in a minority in a number of districts. No line of separation between the various groups could be drawn. Minority problems therefore became inevitable, and two actually arose: the Ukrainian and the Jewish.

The central marsh region had been left entirely undeveloped by the Tsars and the northern regions were not much better. There was no educational system, and the people were almost entirely illiterate.

Throughout the eastern region almost all the land had been held in large estates, the owners being Poles. The peasants' land was farmed on the strip system, each man's strip being allotted on the principle that each was to have his share of good and of bad land. The system was of low productivity and incapable of improvement. Further, at the peasant's death his land was divided between all his sons¹; it was not confined to the eldest as here. Dowries, which could be livestock, had to be provided for daughters. So the peasants became poorer and the inevitable results followed: early marriage, high birth-rate, high rate of natural increase, greater pressure on the land, further fragmentation of the holdings. The situation was worst in the south, and this, coupled with the legacy of trouble fomented by the Austrians, led to the Ukrainian problem.

The Poles began quite rightly by improving the land situation. The peasant is not interested in political theories or systems of government: he tolerates almost any system that gives him sufficient land and peace to cultivate it. But trouble is always likely when fragmentation has gone too far. Land reform was therefore started in four directions: consolidation of the peasant's holdings by exchange of strips so as to bring each man's holding into one compact piece; opening up of Government land and breaking up of large estates; reclamation (usually by drainage) of land which was either waste or not fully utilized; and abolition of embarrassing liabilities. In general estates might not exceed 300 ha. of cultivated land in the eastern provinces or 180 ha. elsewhere, forest and water not to count. The breaking up was hastened in the eastern provinces by the Russian Revolution. In the six eastern provinces 2,230,000 ha. had been consolidated, 1,162,000 ha. had been parcelled out into 305,000 farms, 209,000 ha. had been improved by drainage, and liabilities on 264,000 ha. had been liquidated, all between 1920 and 1936.

More amelioration work was done in Polesie and Wołyń than in any other province in Poland except Lublin. The breaking up of the large estates was completely altering the social structure of the region, but it was vigorously pursued, especially by Mr. Poniąkowski as Minister of Agriculture. As the large estates often obtained higher yields per hectare than the small ones, the breaking up meant a fall in yield per unit area. But the gain in production caused by consolidation and amelioration more than offset this loss and the total production showed a substantial increase in spite of the fall in average yields of rye and wheat.

¹ This is general, except that a few of the larger estates, e.g. the Radziwiłł, Czartoryski, and others were entailed.

All Poland: Production in thousands of metric tons and yields in quintals per hectare

	Rye		Oats		Wheat		Potatoes	
1909-13 ..	57.11	11.2	28.14	10.2	16.78	12.4	247.9	103.0
1932-36 ..	65.26	10.9	25.72	11.6	19.49	11.2	317.1	114.0

Cattle had increased from 9.06 million in 1929 to 10.20 in 1936; pigs from 4.83 to 7.06; sheep from 2.49 to 3.02. The average yield of milk per cow rose from 3022 to 3172 kg.; that on farms under 50 ha. from 2433 to 2560; on farms over 50 ha. from 3219 to 3337 kg. (1 quintal per hectare is 0.8 cwt. per acre; 1 kg. of milk is 0.22 gallons.)

Further improvement was effected by means of education and advice based on research carried out at Experimental Farms and Research Stations. All children up to the age of thirteen had to go to primary schools. Agricultural schools were set up for boys of seventeen to twenty, and some for girls; by 1936 there were in all Poland about one hundred and sixty of them, each with 10-50 ha. of land, and taking forty to fifty scholars for an eleven-months' course. About ten Rural Universities on lines of the Danish Folk Schools, provided short courses for adults, and also a twelve months' course (described in *Wiejskie Uniwersytety Ludowe w Polsce*, Warsaw, 1938), and were started from the well-known Lyceum Krzemienieckie in Wołyń.

The advisers were centred at an experimental farm, and besides giving advice to the farmers arranged periodical visits so that new methods could be demonstrated. Special ways of approaching the peasants were adopted, including the so-called 'Teams of agricultural preparation,' and the 'Popular Halls'; altogether some thousands of adult students were enrolled. In addition Community Houses were set up as centres of social life in the villages.

The agricultural system was also improved. The chief product had been grain. Unfortunately prices fell disastrously during the crisis years of 1930-33; it was estimated that agricultural earnings fell by 60% (Curzytek, *Puławy Monographs*). Peasants were therefore encouraged to carry their production a stage further; instead of selling rye at 15 zł. per q., to improve their grass and feed their grain so as to produce cattle at 65 zł., or pigs at 90 zł. per q. live weight, or butter at 280 zł.; further, the animals should be worked up into finished products: bacon, and sausages.

Credit and marketing had been almost entirely in the hands of the Jews, who lent money and bought the bulky commodities, the grain, potatoes, cattle, and pigs, and transported them to the towns; while the women took the smaller articles, the eggs, poultry, fruit, honey, and anything else they could carry to the nearest market and sat down in the market-place awaiting customers. This made one of the most attractive pictures of country life throughout Poland. The peasant women are very sociable and have a keen sense of colour; the market was always bright and animated, and they clearly enjoyed it and would not lightly give it up.

But the Jewish middlemen were not popular. They never competed one with the other, indeed it was said that the rabbis would allow only one trader per village, and the peasants suspected that they were not being sufficiently paid. The difficulty was met by the establishing of Cooperative Societies, the most popular being the Credit societies. Consumers' societies supplied

goods, but some at least would take peasant produce in exchange; and Dairy or other cooperatives took the peasants' raw materials and worked them up into finished products, converting milk into butter or cheese, pigs into bacon, grading and packing eggs. All three kinds were substantially increasing in membership; for the years 1928 and 1935 the figures were, for all Poland: Consumers' Societies 2022 with 252,000 members in 1928 and 2586 (319,000) in 1935; Dairy Societies had 1430 with 211,000 members in 1928 and 438,000 in 1935; Credit Societies had 3535 with 762,000 members in 1935. The Agricultural Consumers' Societies were mainly Ukrainian, but the Credit and Dairy were mainly Polish.

But there still remained more population than agriculture could carry. In the old days there was much migration to the Americas, but that had been stopped, indeed there had been some reversal of the process and some repatriation had occurred. Industries were therefore developed and manufactured products exported instead of raw materials. The Kwiatkowski Reconstruction Plan was put into operation in 1936. Worked wood and not only raw timber was sent from Polesie, Wilno, and Lwów.

Eastern Galicia had two sets of products. Potash salts were mined at Kałusz, and the output was increased from 14,000 tons in 1913 to 434,000 tons in 1936, and 560,880 tons in 1938. Oil and natural gas were worked in the Lwów and Borysław regions, although the depth of the wells (1500-2000 m.) made competition difficult with Romania, where they are 150-500 m. deep; nevertheless much development was effected and about 500,000 tons of paraffin were obtained in 1936, while the natural gas was piped to Lwów.

Great progress had been made in dealing with the transport problem. No reparations money was available, yet the port of Gdynia was built: the railways were reconstructed and put on to the European gauge; bridges and culverts were repaired. Roads were made, and this was often difficult because in the north only moraine stone is available, and in the south only the granite quarries of Wołyń and of the higher country farther south. The power problem was also being dealt with and the supply of electricity was increasing. The standard of housing was being improved with a view to deal with the overcrowding which was a marked feature of the villages and especially of some of the towns.

Economic progress had been good up to 1928 but was greatly set back by the crisis years of 1931-33 and recovery was very slow, but by 1938 it was perceptible. The villages seemed happier and there was life and movement in the towns. The chief internal troubles were the minority problems: the Ukrainians and the Jews. The Ukrainian trouble was kept alive by outside propaganda, otherwise it looked like subsiding as the result of improvement in the land situation, and the Polish recognition of Ruthenian right to free national development, the establishment of Ruthenian schools, Ukrainian Cooperative Societies, and measures of local self-government.

Looking back over the ten years it is clear that Poland has very faithfully dealt with these eastern provinces. Remarkable progress was made and more seemed to be coming. The difficult land problem had been well attacked, the terrible poverty of the peasants had been mitigated, and the way lay open for steady economic improvement. Education was everywhere being made

available and illiteracy was fast disappearing; the educational ladder though not complete was nevertheless developed and peasant children were in fact stepping up to higher posts. Reconstruction was going on, natural resources were being developed, the younger generation were genuinely trying to help the peasants and to make them realize their share in the country and its government. Had Poland been vouchsafed fifty years of peace a satisfying degree of comfortable life would have been attained; not great material riches, but something much more valuable; good standards of culture and civilization.

DISCUSSION

Before the paper the PRESIDENT (the Rt. Hon. Sir GEORGE CLERK) said: 'This afternoon Sir John Russell is going to tell us about Reconstruction and Development in Eastern Poland. He travelled much in Poland and Russia in the years before the outbreak of war, and no man is better qualified than the Director of the famous Rothamsted Experimental Station to give us an authoritative account of a country in which the improvement of agriculture was playing so large a part.

Sir John Russell then read the paper printed above.

The PRESIDENT: We are privileged to have with us this afternoon H.E. the Polish Ambassador and Foreign Minister. I wonder whether His Excellency would add a word or two on the interesting paper we have had from Sir John Russell?

H.E. THE POLISH AMBASSADOR: After such an interesting lecture there is little I can add and I do not feel that I can trespass on your patience. I should however like to remind you that Sir John Russell is one of the great experts on the part of the world he has described. He very modestly did not speak of the particular work he was doing in Poland when each year he took a certain number of students from England to that country to see what was being done in connection with agriculture and to visit our schools of agriculture. I can only say that when Sir John speaks in general terms of the efforts made to improve agriculture in Poland he certainly speaks as one who knows.

In a lecture so comprehensive and interesting Sir John has necessarily only been able to cover part of the ground. He mentioned the backwardness of the country; that it was undeveloped, and at the same time praised the efforts of the Polish Government to raise the standard of life of the people and to raise the country as a whole to a higher mode of life. He was not able to go into the reasons for that backwardness which of course are closely linked with the history of Europe as a whole and of Eastern Poland in particular. There is no doubt that the Polish Government was trying very hard to develop and to improve the standard of life in all its aspects. Many items other than agriculture came into the picture. For instance, the development of the network of roads which, traditionally, always very poor, had been much improved. On the whole, it was somewhat paradoxical to find some of our high roads in the east were better than those in the central and western parts of Poland. Much was also done by way of development and improvement of the towns; new schools were built and new industries created, such as the flax industry mentioned by Sir John; and even the export of certain products was attempted with some success. In addition, better livestock was available and the newly formed factories were able to supply, in fairly good condition, the fertilizers needed for the improvement of agriculture, although the fertilizers were not used to the extent they should have been.

That, in a way, is an idyllic picture of peaceful effort and of constant although slow improvement. All that was put an end to in a violent way, and the whole of the work will have to be begun anew. When I consider how slow and how difficult was the beginning of that work, and when I recall the tragic history of those lands, the backwardness and the slowness are explained. Perhaps our children will be more impatient, seeing how in many parts undeveloped and primitive life has remained. They perhaps may forget even in our country, certainly in foreign countries, the strong, tragic, and serious reasons there were for that backwardness. I hope that in spite of the incredible disasters, in spite of the destruction of so many houses and towns, many of which have disappeared or been largely changed, fortunately the large towns, such as Lwów and Pińsk, have suffered less than some others, and many of the private houses will not for ever cease to exist. I hope that in spite of the unspeakable disaster and the great wreckage all over my country, we shall before long be able to re-start our work, and perhaps in better conditions and with more prospect of stability than during the last twenty years. I trust that when the war is over we may find in Great Britain, and in the west in particular, the source not only of moral but of material help which will speed up our important work.

The PRESIDENT: His Excellency has made most illuminating comment on a particularly interesting paper on a part of the Continent about which, considering it is in the heart of Europe, many people know extraordinarily little. Some of you may have known something about Eastern Poland, but to me it was an almost *terra incognita*. I am sure we all feel grateful to Sir John Russell for his most interesting paper.

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COLLECTIVE FARMING IN RUSSIA AND
THE UKRAINE

AT the outset I must remind you of a few geographical facts in regard to European Russia. It is a vast rolling plain, with no mountains except at its edges, but it has a backbone of higher land in the centre so that most of the rivers rise here and wind slowly north, south or east to the sea. Moscow at the centre owes its rise and development to the fact that it is near to all of them. The rainfall (including the snow) is highest in the west central part and falls off as you go to the south-east, but it is nowhere high by English standards: not more than 25 inches. In the wetter part there is much forest; coniferous trees and birch in the north, more deciduous trees in the centre and to the south, but with much marsh. To the south-east where the rain suffices for grass but not for trees, there is the black earth and the steppe, and still further eastwards the steppe becomes more arid in character. The forest and the steppe have given a distinctive character to Russian life, just as its rivers have played a great part in shaping its history. It is impossible to convey any adequate impression of the vast size and almost endless solitude of Russia: even in 1935 only about 6 per cent. of the land of European and Asiatic Russia was in cultivation, the rest was mostly wild.

From early times the Russians adopted a system of agriculture very much like the old three-field system, with its scattered strips common in

northern Europe. Alongside a feudal system very different from ours were the peasant Communes who held in common the land allotted to them, periodically redividing it among themselves. The peasants' share grew steadily, and they always wanted the whole of it; they had an unchangeable belief that the land belonged to the men who tilled it. There is an old peasant saying: "My back belongs to my master but the land belongs to me"—and this in spite of another: "The peasants back is made to be beaten."

The agricultural system had two grave defects: it was incapable of technical improvement and the scattered strips involved much waste of time. Stolypin in 1910 had arranged for consolidation of the holdings and for the establishment of peasant farms with state loans to finance improvements, the logical outcome would have been the Danish co-operative system. But before his reforms could achieve results the war came on and then the Revolution. The peasants joined in the liquidation of the landlords believing that at last the whole of the land would be theirs. They were bitterly disappointed when they found it was not.

One of the earliest of the new activities was the establishment of State Farms. They were on factory lines. The farms were very large, so as to secure all the advantages of large scale management and allow of the fullest use of machinery and of scientific methods; one of the best known was Gigant on the Don Cossack steppe about 120 miles due eastwards from Rostov. When I first visited it in 1930 it exceeded half a million acres—considerably larger than Leicestershire—and, as usual in those days, the Director was a politician, the justification being that the purpose

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of all the national activity was the founding of a new order of society, and the detailed work of running a farm was only incidental thereto. He had, however, a Technical Adviser but the Director need not accept his advice. I still remember the long and impassioned speech on the principles of Marxism and Communism to which I had to listen on a hot day in August, with the camera men and their dazzling searchlights actively at work the whole time. The area proved too big. Another one I visited was half the size—but still nearly as large as Bedfordshire. The workers, instead of living in separate cottages, were housed in great barracks; they had their separate bedrooms, but a common dining room; there was also a large meeting room—a sort of theatre. As everywhere in Russia there were political slogans in huge letters on scarlet banners hung up on the walls, with portraits of Lenin, Marx and Engels. On my second visit in 1934 the slogans were a little different: "Practice self criticism; do not judge by looking at other people's faces;" "Develop Party Politics" and the portraits had changed, more prominence being given to Stalin.

But the peasants never really liked these State farms and they were not developed. There was for a time a period of what was almost peasant proprietorship which the peasants liked much better. It was the so-called "New Economic Policy," dominated by Bukharin's slogan of 1925, "Peasants, get yourselves rich!" But it was theoretically objectionable so was given up. It was replaced by a new method, Collectivization, introduced in 1927 and actively developed from the spring of 1929; the method is attributed to a

Ukrainian. The entire village and all its agricultural land was to be run as one farm. All land divisions were to be obliterated and the whole area, which might be 2,000 acres or more—in the south and the Volga regions it might be up to 10,000 acres—was divided into some half-dozen fields, to correspond with the rotation; the whole village population were to come in as workers. No wages were to be paid but all their possessions were to be pooled and all the produce shared after the necessary outgoings, including the Government share, had been met.

There was at first tremendous opposition on the part of the peasants. They understood the idea of collective ownership of land but not of live-stock. Those who had worked hard and built up a little farm, with a few animals and implements and stocks of seeds, greatly resented having it all taken away. Further, the poor harvests of 1931 and 1932 and the many requisitions of grain, left them faced with hunger and rather than give up their animals they killed and ate them, doing much other destruction; in short, they adopted the "scorched earth" policy, the Russian peasants' traditional method of dealing with a hostile situation. The Government took a strong line and great numbers of peasants were removed and disappeared: how many will never be known; in the unequal struggle they lost as they were bound to lose. But Russia came near to starvation and in the end Stalin called off the fight. The fall in numbers of live-stock was enormous.

Several methods were adopted in trying to reconcile the peasants to the new order. Probably the most effective was the introduction of the tractor. The peasants were shown what it could do;

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how it could plough in one day far more than any of them could have done in a week, and so the tractor was sent round adorned with a banner and accompanied by a shock brigade practising all the arts of propaganda in which the Russians are such past masters, and compulsion as well. The tractor became much more than an implement; it became the symbol of advancing civilization—"overcoming the age-old backwardness and poverty of agriculture" to quote one of the slogans. The Russians, even the peasants, have an innate respect for what they call "culture": the connotation is much wider than in English and it includes all the amenities and decencies of civilized life. You not infrequently find notices telling you to use a particular appliance "in a cultural way"; you cannot insult a Russian more deeply than to say that his actions are "uncultural." The propagandists were very zealous Communists fired with missionary zeal and what seemed to an Englishman almost fantastic enthusiasm. You will find the full story in Sir Bernard Pares' remarkable little book on Russia, and dramatic accounts in Maurice Hindus *Red Bread* and in Sholokov's *Quiet Flows the Don* and *Virgin Soil Upturned*. I knew several of the propagandists: one, a White Russian peasant, who felt she owed everything to the Revolution, was almost the living image of the chief character in Sholokov's story.

What, however, most sharply distinguished the new system was that production was planned: the first five-year plan ran from 1928 to 1933; they were in the third when war broke out. Individual farms were not left to grow what they liked, they were told what they must grow and how much of

it they were expected to produce. The plan is drawn up at the State Planning Committee (Gosplan) in Moscow, it allocates the respective shares to the different regions and notifies the governments of those regions. These can make suggestions for changes which are duly considered but the final decision lies with Moscow. The regional Government allocates the plan to the different districts and these to the different farms. Here again discussion is permitted but once the final decision is made it must be accepted. So each Collective Farm knows what it has to do.

The plan for 1937 and for 1941 in comparison with the realization of 1938 are given in Table I.

Million ha. 1 ha = 2.47 acres.	Plan for 1937	Realized in 1938	Plan for 1941*
Total area sown	138.9	136.9	157.0
Grain	104.0	102.4	111.0
Vegetables and Fruit		9.4	11.4
Fodder crops	13.7	14.1	22.5
Technical crops	11.0	11.0	12.0

Total area of U.S.S.R. 2109 million ha.

TABLE I. Areas planned for sowing in 1937 and 1941 and actually sown in 1938.

The 1939 plan shows the relation of Collective to State farms and gives the proportion of fallow: the figures are in million ha.:—

	Collective Farms	State Farms	Total
Spring crops	76.14	7.51	83.65
Winter crops	34.30	2.28	36.58
Fallow	29.63	3.18	32.81
	140.06	12.97	153.04

*Voznesensky, N. Economic Results of the U.S.S.R. 1940 and Plan for 1941, Moscow 1941. It is not clear whether the boundaries are the same as in 1938.

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Old 3 courses	Williams' proposal*	Modern 6 or 8 courses			
		Dry Regions (Saratov)	Moister Regions (Gorky)	(Ukraine)	Old Norfolk
Fallow	Fallow	Fallow	Fallow	Grass	Clover
Winter Rye or Wheat	Winter wheat	Winter wheat	Winter wheat	Winter wheat	Wheat
	2 years Lucerne and grass	3 years Lucerne			Culti-vated crops
Spring corn	Spring wheat (hard)	Spring wheat	Spring corn	Spring corn	Spring corn
	Spring wheat (soft)				
	Sun-flowers etc.	Sun-flowers	2 years grass	Culti-vated crops	
	Wheat		Flax	Millet	
	Millet etc.		Culti-vated crops		
			Spring corn		
Percentage of					
fallow 33	11	14	12.5		
grain 66	44	28	37.5	40	50

TABLE II. Old and new rotations in U.S.S.R.

*Separate fodder crops are grown for the animals.

Great efforts are made to utilize science as fully as possible. Even before the Revolution Russia had possessed good agricultural colleges and agricultural research stations where important investigations on soil formation and soil classification had been carried out. After the Revolution these were greatly expanded and new ones were added.

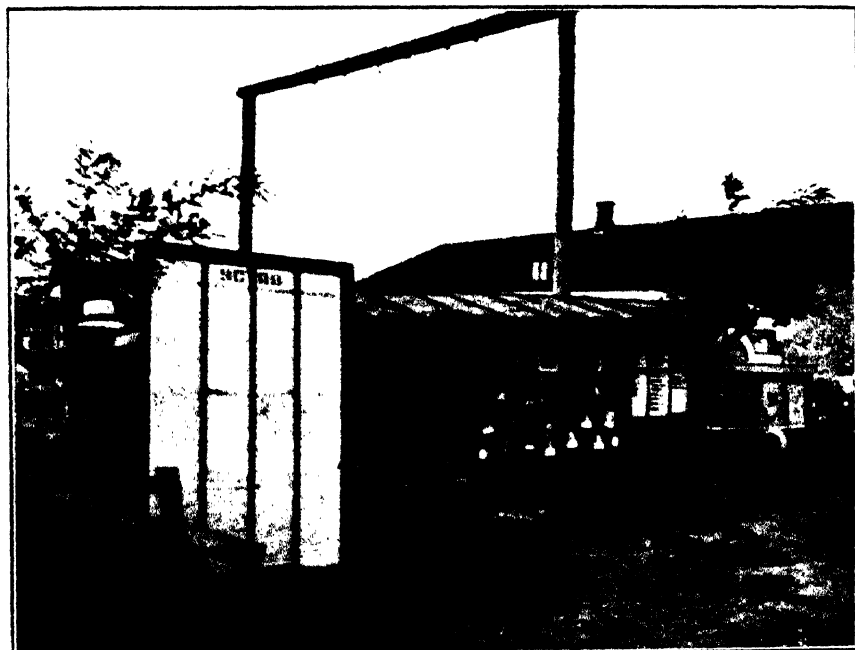
Elaborate soil surveys were organized under Polynov at the Dokuchaiev Institute, which was founded at Leningrad but transferred in 1933 to Moscow, and soil maps were prepared for use in drawing up the plan for agricultural production. Investigations on the manuring of crops were made by Prianishnikov and his staff at the Timirazev Academy, and successful search was made for natural deposits of potash and of phosphate; nitrogenous fertilizers were made synthetically. Rotations were studied by Williams—son of an American engineer and a Russian mother, whose knowledge of English agriculture convinced him that grass and clover and cultivated crops must be included as they had been in England. He worked out basic rotations which have been adopted and modified in the different regions; it has been necessary to keep the fallow in most regions, but occasionally it could be replaced by crops where the rainfall is higher. The old three-course rotation was thus changed as shown in Table II.

These rotations are not yet widely adopted; if they were the percentage of fallow would have been reduced to about 14; as it is, it is 20 to 25, which, of course, is a great improvement on the 33 per cent. of the old three-course rotation.

In addition to the central Institutes, there are



A. The Party Representative in his Office on a Collective Farm.



B. Ukraine Collective Farm—The Rules and Regulations. Photographs of Stakhanovites and Wall Newspaper.

large Institutes with research staffs studying the most important problems of the different regions. One of the best known of these is (or was) at Odessa, where Lysenko did much of his work on vernalization and on the production of new varieties of wheat and cotton, not on Mendelian but on Marxist lines. He comes of a peasant family and was greatly honoured as the embodiment of the Revolution—the peasant become Academician.

The Saratov Institute is another example. It was started in 1929 and has expanded so that in 1939 it had a staff of 118 senior scientific and technical workers and 280 assistants, with a budget of 3 million roubles per annum; still further extensions were contemplated. Its principal work is the struggle against drought and the staff are daily reminded of this by a huge scarlet banner hanging up in the Conference room and bearing Molotoff's slogan "The Bolshevik struggle against drought is the organized struggle on Soviet lines for the harvest." The average rainfall of the region is only 12 ins. per annum and often there is less or it is badly distributed and then comes famine. Drying winds, dust, and mist also do much damage. The chief crops are wheat and sunflower, the latter gives an oil much used in cooking; it makes good to some extent deficiencies of other fats. Suitable resistant varieties of both crops have been obtained by cross breeding (in-breeding has been given up). Great efforts are being made to find out how to grow potatoes in these naturally unsuitable conditions. The potato crop is being extended wherever possible, its dietetic value being fully recognized.

Associated with these Institutes are advisers

who tour the countryside and keep in touch with the collective farms. These advisers have a local centre, called a "hut" laboratory, usually a cottage fitted up as a museum or showroom, with pictures of common diseases and pests, specimens of fertilizers, improved varieties of seeds and other things about which the adviser would talk, and generally speaking, anything likely to interest or help the peasants.

The organization within the Farm is comparatively simple. The members of the Collective meet and elect a Committee and a Chairman, who, however, must be acceptable to the Party, he receives a higher rate of pay than the others. The ordinary members of the Committee are not paid, however, but regard their service as a social duty. The Committee decide how the plan is to be carried out, they cannot modify it but only discuss how best to do it. They divide the workers into groups called "brigades" (military terms were used from the outset), each under a leader and they allocate the tasks. The numbers of workers per 100 acres are usually much higher than those to which we are accustomed.

There is also another official whose role is less easy to describe: the representative of the Party (Plate 1A). When collectivization began Stalin had declared that the Party "can no longer confine itself to individual acts of intervention in the process of agricultural development; it must take over the leadership of the Collective farms." Those I have met were not technical men; they have to see that the plan and also the decisions of the Party are carried out. In 1934 their influence was very great, but in 1937 and especially in 1939, I got the impression that it was less; also the func-

tion had changed;* the office had, of course, the usual photographs: Stalin, Molotov, Kalinin but there were also exhibits of technical interest. Very few of the members of the Collective are members of the Party, the idea is to keep it small and select so as to ensure obedience and efficiency. This is true all over Russia; by far the greater part of the population call themselves "sympathizers," and many of my Russian friends have assured me, especially in the years before 1937, that this was the safest line to adopt. A member of the Party might do well for a time but if he got "purged" it was bad for him. The young people are advised to become Pioneers (aged 10-16) and afterwards Komsomolisy (ages 16-21) but only few will become Party members.

Rules and regulations are fairly numerous, and are posted in a prominent place for all to see. (Plate 18.) An outlet for the farmer's universally admitted right to grumble is found in the so-called "Wall Newspaper," a sheet written by hand and hung up prominently, where complaints may be voiced and offenders against the rules may be reproved. Thus you may read that Ivan Feodorovitch drinks too much and so doesn't get his day's work properly done; that Boris Dimitrievitch is an idle fellow and must mend his ways. In 1930 the wall newspapers had been very serious and I have known a Professor put in considerable trepidation because a student had written saying that his lectures were dull and no one could learn anything from them. But as time went on the comic sketch and the humorous article began to appear.

Every effort is made to increase output. As

* See L. H. Hubbard, *Economics of Soviet Agriculture*, pp. 157 and 319 for a full account.

between different farms and regions "Socialistic competitions" were started. In his speech at the 1934 Plenum of the Communist Party of the Ukraine, Postyshev declared that "we have aroused a tremendous war of socialist competition between regions and districts, collective farms and brigades. We discovered thousands of heroes of collective farm production." And in the wonderful agricultural exhibition in Moscow opened in 1939 there hangs, written in scarlet, Molotov's dedication: "This exhibition shows the whole programme for agricultural improvement and should stimulate competition between farms and Machine Tractor Stations, between districts and regions and republics." A banner is given to the winner of a competition.

Other methods are applied to stimulate individuals to greater activity. Besides the admonitions of shirkers in the wall newspaper, direct encouragement is given to the best workers by publicly exhibiting their photographs. (Plate 1B.) In the earlier days these best workers were organized in special brigades, the "shock brigades" —the *udarniki*— which were called in when work was specially important or urgent. They had certain special privileges. In recent years these specially effective workers are called *Stakhanovites* after a coal miner Stakhanov, who found a way of considerably increasing his output. As they are paid on a piece-work basis the more they do the more they earn. In 1939 the method was being intensified and a farm that exceeded its "planned" output was to receive a bonus on all excess deliveries, so that workers and especially its *Stakhanovites* would receive still higher rates of pay. In consequence one meets with great inequalities of income in Russia.

The payment on the farm is mostly in kind. Various outgoings have to be met. The Government share has to be sent off; there is a very small payment for it. The Machine Tractor Station has to be paid for its services, and provision has to be made for seed, insurance, capital expenditure and such social services as sick and needy, the crèche, etc. Whatever is left is shared among the workers in accordance with the number of "labour-days" they have put in: the classical formula "to each according to his needs" was found unworkable and was replaced by "to each according to his work" now embodied in Art. XII of the Constitution. A "labour-day" is not counted by time but by the job. The Committee decides that a certain job, such as the sowing of a certain area of land or the milking of a certain number of cows, is a day's work and when this is done the worker gets credit for one "labour-day." He can accomplish two or even three "labour-days" in one day; then he gets double or treble pay. The calculation of the remuneration is very complex; it is made with the abacus, a little instrument possible only because everything is done on the decimal system: yields are in quintals per hectare and prices in kopeks and roubles (1 r.=100 kopeks). Some of the figures are given in Table 3.

The system involves the peasant bearing the loss due to season or diseases, and in consequence even on the same farm the payment varies from year to year. In any given year the peasant never knows what he will receive until the harvest is in and the accounts all paid. Naturally during the year he has to draw advances.

It is not possible to say how much of the total produce is available for division among the

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	Shpitky, Ukraine			Karl Liebknecht Ukraine
	1933	1935	1936	1936
Grain, kilos	1	1.8	2	2.5
Potatoes „	5	4	10	0.5
Hay „	4	1	1.5	5
Vegetables,,	3	1.5	2	3
Apples „	0.1	—	—	(Grapes 0.5 k. Wine 0.5 l.)
Honey „	—	0.1	0.2	
Cash r.	0.79	0.70	1.10	10

	Steingut	Saratov	Tarasovka Moscow
	1937	1938	1937
Grain, kilos	2	None	None
Potatoes „	—	—	10
Hay „	not measured		3
Vegetables,,	—	—	10
Apples „	3	1	—
Honey „		(Cherries 0.2	—
Cash r.	4.70	5.03	20

TABLE III. Value in money and kind of one "labour-day" in certain Collectives in different years.

peasants. Figures given me on a number of farms vary: some range about 50 per cent.*, but this is still subject to loss on storage which may be considerable.

Troubles lasted for some time. I remember well in 1930 a disgruntled group of peasants who,

*From "Collective Farms in the Second Five Year Plan", a statistical summary issued by Gosplan, it appears that in 1937 the average "Labour-day" rewards per "Dyon" (household) were 17.4 q. of grain and R. 376 in money. This works out at about 30 per cent of the total grain harvest and about 48 per cent of the total money income.

having put the invariable question "Are you a worker?" and received a favourably reply, proceeded to show me a day's ration of bread, already mouldy and smelling badly, and then offered me some tobacco. I said I didn't smoke and they replied: "It is just as well, this tobacco would only make you sick." So also in 1934 there was much discontent. Then in 1937 I saw a marked change. The peasants had always wanted to own the land and this desire for ownership was recognized. By the new Constitution of 1936 the land and all that is beneath it was declared in Article 6 to be "state property, i.e., the property of the whole people," but by Article 8 "The land occupied by collective farms is secured to them for perpetual use, i.e., for ever." As I visited each farm in 1937 I was shown with great pride and with sparkling eyes the Title Deeds recently received, vesting the land in the Collective for ever. The peasants now believed that at last the land really was theirs. The long struggle, first with the landowners and then with the State, seemed to be terminated in their favour. Further, the peasant's desire for a piece of land of his own was granted. Article 7 stated that "Each collective farm household has for its own use a plot of land attached to the house and as individual property—the house, produce animals and poultry." Later decrees regulated the size of the holdings: they vary from half up to one or more acres, according to the region and the type of farming; on these the peasants can grow what they like. Each household was promised a cow, one or two pigs and some poultry. The peasants may dispose of the produce to the Co-operative or in any way they please; there is, in fact, a good deal of selling in peasant

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markets especially by the women. Many found their own piece of ground more profitable than the collective. The peasant's wife and children may help him, but he may not pay any wage; that would amount to exploitation of a man's labour, which is forbidden. You may hire a person to look after your house or your dog if

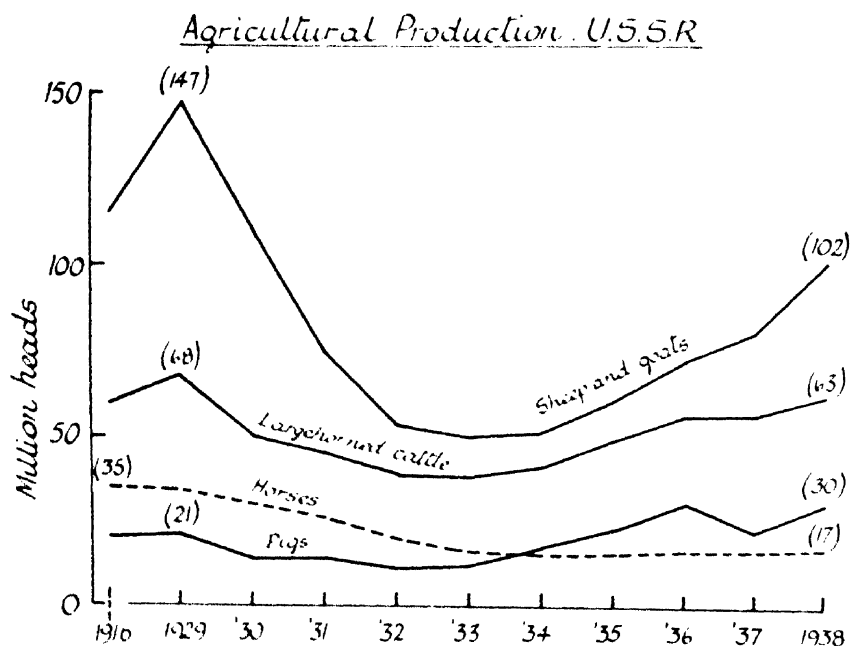


FIG. 1.

you have one, but not to look after your cow because that is an animal for production.

Thus the peasant's total income is derived partly from the Collective, partly from his own plot and sometimes from other labour. The proportions vary a good deal. In the Gosplan publication already quoted the average income from labour-day payments per household in the Collective farms in 1938 was 17.4 q. of grain and R. 376 while the total income was R. 5,843; putting

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the grain at R. 25 per q., the income from the farm is less than 14 per cent. of the total. On the other hand, the percentage distribution of working hours in 1937 was* :—

	Work on the Collective	Work on private allotment	Work outside the farm	Domestic and other duties
Men	67.5	4.0	23.9	4.6
Women	52.8	19.6	7.1	20.5

Whatever the average, some workers instead of putting in the average two hundred labour-days on the Collective, were putting in far fewer and stringent orders were issued that not less than 60 to 100 labour-days per annum (according to the district) must be devoted to the farm.

*As against this in "Communal economic foundations of Kolchoznik prosperity, 1941" it is stated that in the province of Voronezh the ratio of income from work on the Collective farms and on private holdings was as follows:—

	Income: r. per worker per annum		Per cent of income derived from Collective	Distribution of time		
	from Collective	from private allotment		on Collective	on private	other work
Superior farms	5510	597	90	85	14	1.0
Good farms	4267	771	84.6	80	17.5	2.4
Medium farms	3035	849	78	72	22.6	5.4
Poor farms	2080	852	72			

These figures show the impossibility of generalizing about Collective farming. I am indebted to Mr. L. Hubbard for these and other data.

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In one direction, however, the private property took on very large proportions. It has been already stated that the livestock were drastically reduced when collectivization began. The numbers fell till 1933, then slowly rose, but the increase has been marked since the peasants were allowed animals of their own. (Fig. 1.) By 1936 the numbers of animals on the Collective farms were, in millions* :—

Ownership	Cattle	Pigs	Sheep and goats	Land under crops, million ha.
Collective	14.8	6.3	22.75	116.0
Private	25.2	12.9	31.26	9.1
Private as per cent of Collective	172	207	137	8

TABLE IV.

The results of the recent farming efforts in Russia up to the end of 1938 when the last official figures were issued have been: (1) an increase in numbers of livestock so that they had nearly reached the high levels of 1929, pigs indeed had exceeded all previous records; (2) an increase in the area of cultivated land, which fully kept pace with the increase in population; (3) marked increases in the area of fodder and of technical crops; (4) a smaller increase in area of grain crops which represented three-quarters of the whole sown area. The yield of cereals per acre is still dependent largely on the season and it is not

*Kolkhozy vo vtoroi Stalinskoi Piatiletke, 1940. Beside these animals there are others on the State farms, but even when these are added in the privately owned animals are still 40 per cent of cattle and pigs and 30 per cent of sheep and goats.

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certain that any increase has occurred; comparison is rendered difficult by a change in 1933 in the method of estimating the yield; American authorities consider that the new method gives estimates about 5 per cent. higher than the old one for one and the same crop.

The grain results for the U.S.S.R. are given in Table V.

OUTPUT OF GRAIN, U.S.S.R.

	Popula- tion Millions	Total area sown Million ha.	Cereals sown Million ha.	Cereals produced Million tons	Yield quintals per ha.
1913	134	105	94.4	78.8	8.49
1934		131	104.7	88.0	8.54
1935		132.8	103.4	88.7	8.71
1936		133.8	102.4	81.4	8.08
1937	169	135.3	104.4	118.1	11.52
1938		136.9	102.4	93.5	9.28
Increase per cent	27	30.4	8.5	18	

1 q. per ha. = 0.8 cwt. per acre. Biological estimates introduced in 1933. Average yield of wheat in England and Wales 18 cwt., and of oats and barley 16 cwt. per acre.

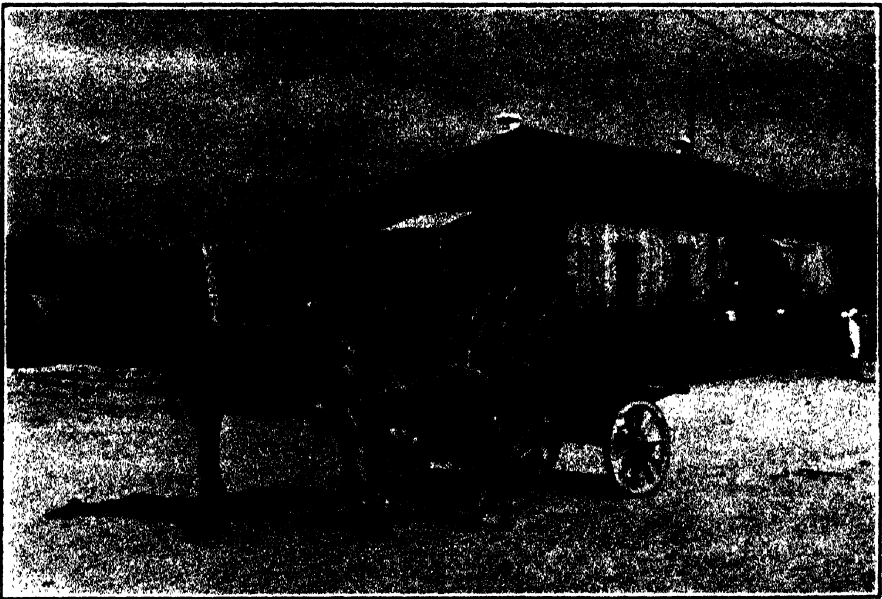
TABLE V.

The villages themselves lack picturesque attractiveness; usually they are built along a road or sometimes round an open space, but it is always an earth road with no side walk, very muddy in wet weather and very dusty in dry. The cottages are small and very simple, made of local materials, wood in the north, wood or whitewashed adobe in

the centre and the Ukraine; thatched with straw or roofed with wood or sheet iron, painted red but soon becoming reddish brown. Iron is safer from fire. In the north there is an attic or garret; elsewhere the cottages have one storey only. Usually there are two rooms and a kind of entrance or large lobby, beds in each room, one room has the brick stove, in the lobby there is a cooking stove, but in the south this is often outside, it is then made of clay. Beyond a table and a few seats there is little furniture, though there may be a kind of dresser or cabinet containing some china. In the Ukraine there may be a trunk holding some of the old peasant embroidered work and shown by the old lady with great and justifiable pride. Usually an ikon hangs in a corner, which, it is explained, is for the old people; there may be a portrait of Stalin for the younger ones; a few faded personal photographs may complete the adornment. Lighting at night is sometimes a difficulty when shortage of fats and oil have curtailed supplies of candles and lamps; a pine splinter may then be used. But many villages have electric light. Usually there is no sanitation. Water is drawn from a communal well operated by a wheel and bucket; naturally this becomes a centre of life and gossip. (Plate IIA). Elsewhere the cottage has its own well with a long pole as lever to lift up the bucket. In summer there are many flies, though a vigorous campaign is organized against them and on the clinics you may see a scarlet banner with the slogan, "Keep away flies: they cause decay and disease," or another, "Keep clean and so prevent disease." There are mosquitoes and various domestic insects. When you have seen a peasant woman combing a girl's



A. In a Volga Village.



B. The Club-room in a Volga Steppe Village.

COLLECTIVE FARMING IN RUSSIA

hair you appreciate the force of Postyshev's demand that "hygienic baths and hairdressing shops in the villages must occupy an important part in our Party organization." Naturally one hears of dysentery, enteric, malaria and, at times, typhus, besides stomach troubles. Where there is a local hospital the doctor, often a woman, is kept very busy. The very young children often look sickly; those that grow up, however, look well and in summer they get much sunshine. There are lots of them, very friendly and accessible, very fond of being photographed. The Government encourages large families and gives a bonus of 2,000 roubles for the seventh child. But it is only in the country you see them; the town dwellers, like our own, usually have small families.

The women commonly wear a dark skirt and white blouse with a white cotton square tied round the head, but the younger ones wear a printed cotton frock and a printed or embroidered square tied at the back of the head. The embroidered peasant frocks and saraphans of the old days are out of use and deemed old fashioned. The men commonly wear tunics, trousers and peaked caps; some are bare footed, some wear bast shoes, others canvas or leather shoes; the smart young men in the Ukraine wear white tunics with embroidered edge and the high Russian boots. All clothing, however, is of very poor quality; the clothes of my English friends were always stared at with great curiosity. One sees few old people either in villages or towns; Russia always impresses the Western visitor as a land of young people. The survival rate after 50 is not as high as in the west.

Each house is in its own piece of land, separated

by a rough pallisade from the road. Outside the house is the pile of fuel; always local material, it may be peat but is often straw briquettes. One sees but few flowers, although the Russians like them; there are vegetables, however, potatoes, cabbages, tomatoes and little cucumbers; these one finds and eats everywhere, and often the big water melons. There are also poultry, one or two pigs and the cow, but usually no dog and no cat; you can travel far in Russia and meet few of either. The peasants' dietary is simple, mainly black bread, millet porridge (Kasha) and the vegetable soup known as "shchi"—made with much cabbage, some onions and other vegetables; or "borshch" made with beetroot. Sunflower oil supplies the fat, but some pork is eaten; sometimes you see tinned meat, or on the Volga, dried fish. Tomatoes and little cucumbers are much liked. Apples are the only fruits one sees as a rule; they are widely grown but not usually well grown; there is, however, good research on this subject. In the communal kitchen one often meets a compote made of fruit pulp. Tea and coffee are too dear for common use; on the Volga hot water with a piece of apple in it is often drunk. As alcoholic drink there is kvass, made from fermented black bread and when well made something like fortified ginger beer, and the universal vodka—a very potent spirit of which a good deal is consumed. One notices this in the provincial towns at night.

The administrative centre of the village is the Chairman's office, usually the cottage of a former kulak, built of brick and somewhat pretentiously decorated. Here one is taken on arrival at the village. Of course you cannot wander about in

COLLECTIVE FARMING IN RUSSIA

Russia as we do here; the visit has to be arranged well beforehand, no local official can give the necessary authority and higher officials are not easily accessible. In consequence of this difficulty I could not in 1939 obtain permission to visit any grain farm in the Volga region. In the office the President and some of the Committee receive us; the book-keeper is there with his abacus. On the walls are the portrait of Stalin, a print or some chart likely to interest or stimulate the village; it may be the list of yields or a diagram illustrating the different rates of work; a slow brigade represented by a tortoise, the better brigades represented in ascending order by a donkey, a bicycle, a train, an autobus and an aeroplane. Something of the old kulak's possessions may remain; a walnut clock of Victorian design but long since stopped; a very poor picture; I have even seen a book left by the former owner, the German manager of the estate; it was a Brokhaus Lexicon, with pictures on the inside cover drawn by his children— all long since "liquidated" like himself.

Another communal building is the Club house (Plate 11B) for the Russians are very sociable and gregarious; here there may be a library, a radio set and a gramophone. The Russians dearly love these; there are scarlet slogans advising you to listen-in. The loudspeaker works almost incessantly on the Volga steamer, in the long distance trains, in the city parks and elsewhere; noise never disturbs a Russian. The accordion and balalaika still survive. Then, too, there are facilities for lectures; these in summer afternoons are in the open air, and the lecturer is sent down by the Party. There is no complication about conflicting points of view; only one Party and

only one point of view. In going round an exhibition in Moscow with a few friends, one of them, a distinguished student of Russian history, was giving us some explanations in a very quiet voice, but was at once stopped by the attendant ; only the official guide could explain. The Russian is eminently teachable and has great respect for teachers and especially for professors: in the villages I am always introduced as an English scientist, a specialist in soils, whose books are used in the Russian agricultural institutes; then comes the question, "Has he written anything about collective farming?" Technical books are very widely read in Russia.

The Soviet Government has done a great deal for the development of education both of children and of adults. For the small children there is always the crèche, in charge of a very kindly looking peasant woman. From 8 to 15 they go to the so-called seven-year school of which every village usually has one, or at least access to one, though the buildings may be as yet inadequate. There may be only three class rooms, one quite small. At first the instruction in the schools was related to the local industry, but that is now altered and the schools are on a uniform type of curriculum which is "cultural" not vocational. In the towns the "ten-year" school is now the standard. By 1939 the educational ladder was pretty complete and a bright child had a good chance of getting to the University; this was very different from 1930 or earlier, when only the Party ticket or proletarian birth would admit. I have known young people who could certainly have taken full advantage of a University education and knew it, but were refused; they

remained always disappointed and with a bitter sense of frustration. Even by 1937 ability counted for more than birth or politics and by 1939 the change was complete. The Universities were overflowing; one of the professors told me that the total number of students in the Russian Universities was above 600,000, and that at the larger Universities of Moscow and Leningrad there were ten applications for every vacancy. Many study science and engineering, others, especially women, study medicine or wish to become teachers. German, French and English are widely taught, yet it is most unusual to find any young people who can speak a word of any of these languages, in marked contrast with the older people of culture, many of whom spoke one or more of them with ease; the women often spoke French and the men German. I have often met German-speaking peasants, descendants of German immigrants of bygone days who, as long as they kept their old religion, kept their Biblical German language. But all that is now going and only the few specialist guides and translators can, as a rule, speak any language but their own. I asked some of my University friends why this was and received the reply: "Our education is cultural, not practical."

On the technical side the immense leeway is being made up. It is hard for us to realize what a colossal task this has been. In the old days Russian workmanship was proverbially bad. It is still often stated that the Russian is a poor mechanic, that maintenance of machinery and buildings is inadequate, that tractors and motors are not properly cared for and that many of the tractors are out of commission. But one

must remember the enormous difficulties. Very few of the present generation had, as children, any mechanical toys, apart from some very ingenious wooden toys made by the peasants. Very few even now possess a bicycle, in many villages you see none and I remember once being kept waiting some ten minutes while a bicycle was fetched to show that this village really had one. There are official cars, but hardly any private cars or motor cycles and few taxis, no visible garages, nowhere where a boy can grow into the idea of machinery. In the hills of Georgia I have even met a man who assured me that until he was 21 he had never seen a wheel. Even now, children's mechanical toys are scarce and dear; there are, of course, no cheap 6d. stores, and a very poor toy may cost 3 to 12 roubles. But there has undoubtedly been an advance; the number of tractors has steadily increased to well over half a million and there are now many tractor drivers, some 25 per cent. being women. The Red Army has been a great educational force and has presumably trained large numbers of engineers and mechanics.

The only large building in the village is the Church, often a brick building in the Byzantine style, now usually converted into a club, or a grain store or partly pulled down for its bricks. It is surprising that the Russian peasant, who is always described in pre-Revolution literature as whole-heartedly religious, should apparently have dropped his religion so completely. I talked once about this with a peasant girl who had been through the famine of 1921 and seen her family die, one after the other: first the baby, then the other children and the father, then the mother and

finally she herself had laid down to die but was found by a rescue party. "If you had known" she said, "how much we prayed to the saints to help us and give us food and how terrible it was when they did nothing, then you would understand why we no longer believe in them." The young Russian intellectual, of course, had always been an atheist and claimed that science had displaced religion—visitors to the Tretyakovski Collection at Moscow will remember the ribald pictures of the village priests by Perov in the 1870's. It was the intellectual who furnished the ideas adopted after the Revolution. One meets many of these people at the Universities and elsewhere; their attitude is always that Religion is an antiquated, rather ridiculous superstition, not accepted by enlightened people and the Russian desire to be counted among these is such that the argument carries great weight. To my question, "If a teacher had religious convictions would he be dismissed?" the answer was "No, not if he were otherwise suitable but we should try to teach him better." This combination of ridicule and lure of "culture" (in the Russian sense) has been much more effective than persecution in the struggle against the Church. Religion still survives in Russia; the ikons remain in many of the cottages, there are still churches functioning and people attending the services. Funerals may be either "white" (religious) or "red" (political) and one sees a fair proportion of "whites." I was told, though do not know personally, that many marriages are now not simply civil but religious as well. And there is a Baptist movement both in Poland and in Russia, the depth and significance of which cannot be estimated. The Russian must

venerate something. Watching the long queue standing for hours in the heat and the glare as they wait to pass through the Lenin tomb in the Red Square at Moscow, one gets the impression of something more than respect for a dead political leader. But it is useless to speculate about the Russian peasant—as Turgenev says: “He is like a mysterious unknown: who knows him? He does not even know himself.” There is, however, no doubt about the change in moral standards. Immediately after the Revolution there was a so-called liberation from the fetters of convention that led to considerable licence. Lenin strongly opposed this and the new system was found to be pernicious and unworkable. The revolt against it came from the women and gathered force as the love of sport began to develop; football, volley ball, swimming, above all, parachuting—but not yet cricket or golf. There is perhaps no better tribute to Christian morality than the fact that the Russians have come to it not out of any acceptance of Christianity but because anything else did not answer in practice.

Children no longer receive religious instruction but they are taught to do good work and to lead moral lives. Keen young people devote their spare time to the training of the children, so that the future may be happier than the past. And the most intense patriotism is drilled into them. Stalin’s stirring invocation still holds them “The supreme law of life” for the citizen “is to love his native soil, language and people; to extol the talents, abilities and achievements of himself and his fellow citizens; to hate and reject all that does not make for the greatness of the Soviet Union, the fatherland of fatherlands.”

COLLECTIVE FARMING IN RUSSIA

One of the modern popular songs I heard in 1939 proclaimed that "My country is rich and large; it has fields and woods and beautiful cities, but its chief riches are its people, more free and happy than anywhere else." It is in this faith that the young Russians have been brought up and this, combined with the peasants deeply rooted and almost fanatical attachment to the land, accounts for the superb resistance they are now putting up.

It is probably true that this war would never have arisen had there been in 1939 the same co-operation between Russia and Great Britain as exists to-day. It seems certain that the future peace of the world depends on a continuance of such friendly relations as will ensure similar co-operation whenever peace may be threatened. But friendly relations are possible only on a basis of mutual understanding and respect. We shall always differ in many ways from the Russians in our outlook and mode of life, and nothing is gained by slurring over the differences or pretending that our points of view are the same. Without giving way on any principles which we hold dear we can find much in common with the best of the Russians. Their history has been one long pageant of suffering, yet through it all has shone an intense feeling for humanity, a desire for a better and happier life for those who come after us. It is vividly shown in their literature, in Tolstoi, in Dostoevsky, in Chekhov, in Gogol—even though some of their writings emphasize the gloomy depths of the Russian character just as their ballet reveals something of its dazzling heights. "How much anxiety," says Tolstoi, "how much suffering we go through before happiness is our return"!

SIR JOHN RUSSELL

And it constantly comes out in daily life in Russia: "Things have been bad for me," a workman once told me, "but I don't mind; they will be better for my children." The picture of Russia I always like to remember is that of my friend Sonia among the children trying to ensure that their lives may be happier than hers has been. There surely we have a solid foundation on which fruitful Anglo-Russian friendship can be founded.

[E. J. R.]

Experimental Studies of the Relation between Carbon Assimilation and Stomatal Movement

II. The Use of the Resistance Porometer in Estimating Stomatal Aperture and Diffusive Resistance

PART I. A CRITICAL STUDY OF THE RESISTANCE POROMETER

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INTRODUCTION

IN a previous paper in this series (Heath, 1939) the apparatus and technique used in following simultaneously in the same leaf changes of assimilation rate and stomatal opening were described. For following stomatal opening the principle of the resistance porometer (Gregory and Pearse, 1934) is used. One of the commonest and most cogent criticisms of the porometer method of following stomatal movement is that it does not enable one to estimate the aperture of the stomata. The desirability of calibrating the porometer in terms of actual stomatal aperture is self-evident, and for the purposes of investigation of the relation between carbon assimilation and stomatal movement calibration in terms of the diffusive resistance of the stomata is even more important. In the present paper (Part 1) the resistance porometer method is critically discussed and its use in estimating stomatal resistance to mass flow of air is considered. Parts 2 and 3 will describe the attempts to calibrate the porometer in terms of stomatal diffusive resistance and stomatal aperture. The problems involved have proved to be of some complexity, and although the investigations are not complete in all respects, it is thought desirable to publish an account of them, both in explanation of the use made of the porometer readings in the assimilation experiments (to be published later) and as a basis for further work, especially as experimental work on the subject has had to be suspended for the time being.

Not many attempts to calibrate porometers have been published. Paetz (1930) made use of a Darwin and Pertz (1911) type of porometer and measured stomatal aperture by direct microscopic observation of the living leaf using a Leitz 'Opakilluminator'. Following a porometer reading ten stomata were measured, presumably on the area of leaf previously occupied by the porometer cup. Expressing both the porometer rate and the stomatal width (*Zea mays*), or half the geometric mean of the width and length (*Tradescantia fluminensis*), as percentages of the maximum values obtained, he compared the observed points with a series of theoretical curves in which the porometer rate was proportional to the first, second, third, &c., powers of the stomatal dimensions. He concluded that for 'not too wide' stomata the porometer rate was proportional to the cube of the stomatal width.

Ashby (1931) working with *Pelargonium* and *Verbena* made a comparison of the readings of a Knight (1915) porometer with stomatal areas as measured by Lloyd's (1908) method on strips of epidermis from another leaf. By plotting both sets of data as percentages of the maximum values obtained he showed that the time curves given by the two methods were in good general agreement, although his application of the χ^2 test of significance (Fisher, 1925-38) to the differences of the percentage values was incorrect. Beyond demonstrating this general agreement he made no attempt to calibrate the porometer in terms of actual stomatal aperture.

Newton (1936), in the work at this Institute which gave rise to the present

studies, carried out on *Pelargonium zonale* a preliminary calibration of the resistance porometer in terms of stomatal area as measured by Lloyd's method. Immediately after a porometer reading the cup was rapidly removed from the leaf, and a strip of epidermis was taken from within the area it had covered and plunged into absolute alcohol. Thirty stomata were measured microscopically on each strip. For this calibration Newton made use of only a few leaves at only one time of year, and the results for all the leaves apparently lay on the same curve. He concluded that the resistance to viscous flow was inversely proportional to the 2.76th power of the stomatal area and he gave the necessary constant for the conversion of resistance readings to actual stomatal areas. Preliminary work by the present author indicated that Newton's findings represented an over simplification of the problem and the further investigations to be described later were accordingly undertaken.

With reference to the work of Paetz mentioned above, Williams (1940) has suggested that the empirical result obtained, namely that flow is approximately proportional to the cube of the stomatal width, might be expected on theoretical grounds. If flow through a stoma obeys Poiseuille's formula for viscous flow of a gas through a capillary tube of elliptical section, under given conditions:

$$\text{Vol. of gas per sec.} \propto \frac{a^3b^3}{a^2+b^2},$$

where a and b are the semi-axes of the ellipse. Since for small and medium openings a is small compared with b , a^2 is even smaller compared with b^2 and may be neglected in the denominator. Then since b , the long axis of the ellipse, is almost constant, the volume flowing is proportional to a^3 . The same argument might perhaps be applied to Newton's finding that the flow is proportional to approximately the cube of the area, for since the long axis is almost constant the flow must also be proportional to approximately the cube of the width. It should be noted, however, that in Newton's results the proportionality between flow and 2.76th power of the area extends down to the largest stomatal apertures when a is not very small compared with b .

Attention may now be turned to previous work on the relation between the resistance or conductance of the leaf for viscous flow as measured by a porometer and the stomatal resistance or conductance for gaseous diffusion. Darwin (1916) considered that the stomata should be treated as long narrow tubes, and assumed that diffusion would vary directly with the stomatal area while viscous flow would be proportional to its square, as for a long capillary tube of circular cross section. He therefore used the square root of the porometer rate as a measure of diffusive conductance. Maskell (1928) also made use of the square root of the porometer rate (rate of flow with a Darwin type porometer but corrected for certain sources of error) as 'the closest simple approximation that can be made to an experimental measure of stomatal diffusive capacity'. Only one attempt to solve this problem experimentally has been

published,¹ that of Gregory and Armstrong (1936) who described a porometer—the diffusion porometer—which for the first time enabled direct measurements of the diffusive resistance of the leaf to be made. They gave for *Pelargonium* a curve relating readings of the resistance porometer to rates of hydrogen diffusion through the leaf, the hydrogen being measured in terms of the current necessary for its production. These data were subsequently re-examined by Newton (1936) and found to give the hydrogen diffusion inversely proportional to the 4.74th root of the resistance porometer reading. The present author's experiments give results which are not in agreement with this finding, and possible reasons for this disagreement will be discussed in the appropriate context, but there is no doubt that the diffusion porometer provides a method of investigation of the utmost importance which should be developed and refined to the greatest possible extent.

MATERIAL

A few experiments have been carried out with leaves of *Begonia sanguineum* obtained from Chelsea Physic Garden, but for the main part of the work, both for porometer calibration and assimilation experiments, *Pelargonium zonale* has been used as in Newton's (1936) investigations. Newton was not able to obtain plants grown under comparable conditions for his various experiments, and in order to avoid this possible source of error five clones of *P. zonale* (var. Paul Crampel) were started from cuttings in September 1936. Some leaves were used for preliminary porometer experiments in 1937, and two of the clones (No. 3 and No. 5) were repropagated in September 1937 to give a total of fifty plants for 1938. Both these clones were made use of in 1938 for assimilation experiments and for porometer calibration, and No. 5 was repropagated in September 1938 to provide material for 1939. The old plants of No. 3 were kept on and repropagated in June 1939 to provide material for the autumn, since it had been found that leaf size generally fell off seriously in September in the case of autumn propagated plants. Actually both clones were used for porometer experiments in autumn 1939. By 1940 there were sufficient plants of a single clone (No. 5) for both summer and autumn cuttings and future work will be carried out on this clone.

From the time that the cuttings were taken the plants were grown together under the same conditions,² being kept under glass in a frame with very slight heating. Watering and repotting were carried out at the same times for all plants; all flower buds were removed as they appeared.

The stomata of *Pelargonium* plants grown under glass seem to remain actively responsive to the stimulus of light for a much greater part of the year than those of outdoor plants, and the leaves grow to a greater size. Such leaves also have a smaller number of stomata on the upper surface relatively

¹ Maskell (1928) quotes unpublished work on the subject by Briggs and Maskell.

² Some of the plants were removed to a similar frame at Rothamsted Experimental Station for use in porometer experiments during the autumn of 1939.

to that on the lower; this is an additional advantage for the present assimilation experiments as has been mentioned in the previous paper (Heath, 1939).

The leaves used for experiments were in all cases fully expanded and of more than 4 in. diameter. At the same time leaves showing the slightest symptoms of senescence or insect punctures were avoided. In the case of *Begonia* the youngest fully expanded leaf on a shoot was always selected for experiment. Such leaves measured not less than 6 in. \times 4 in.

The author is indebted to Mr. Robinson and his staff at the Chelsea Physic Garden for the skill and care with which the plants have been propagated and grown.

LEAF STRUCTURE

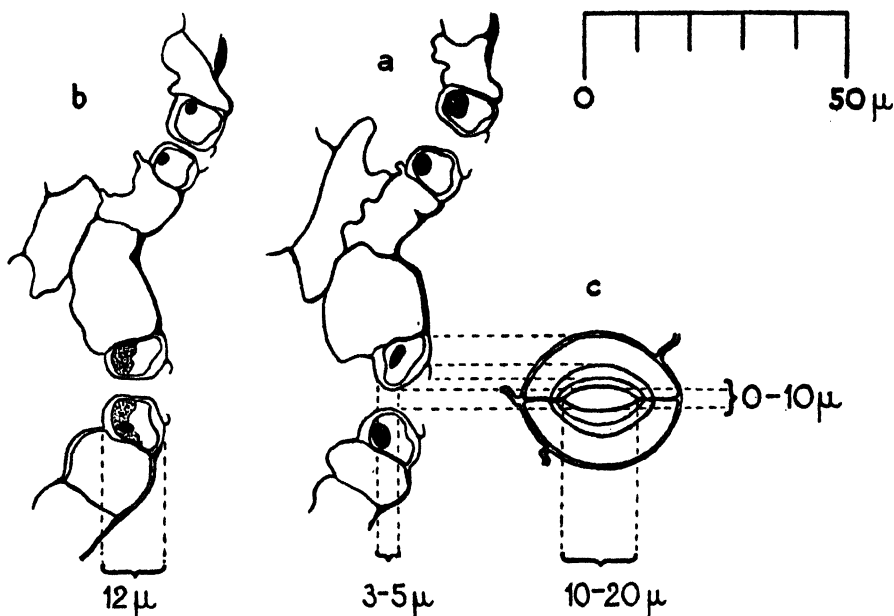
Since use will be made of various dimensions of the leaf and stomata in this and subsequent papers a brief account of the leaf structure¹ in the two species may be found useful for reference.

In the strain of *Pelargonium* used the leaf is mesophytic in type, about 180μ thick with a well-developed palisade layer about 36μ thick. In this layer most of the cells apparently touch their neighbours throughout their length so that the intercellular spaces run almost exclusively at right angles to the leaf surface. Any lateral movement of gas in the leaf, whether by diffusion or viscous flow, must therefore be almost entirely confined to the spongy mesophyll tissue, which including the 'connecting cells' at the base of the palisade is about 100μ thick. The intercellular spaces in the spongy mesophyll account for about 30 per cent. of the area as seen in transverse section. This figure is a mean value from camera lucida drawings of sections 2μ thick of material fixed in Navashin's fluid. The leaf is homobaric and there are considerable intercellular spaces in the mesophyll underlying the very small veins, but it is probable that the main veins provide an almost complete barrier to gas movement either by viscous or diffusive flow. In a large leaf there are spaces up to about 2.5 cm. wide between main veins where a small porometer cup may be affixed. The leaf chambers (Heath, 1939) cross the main veins more or less orthogonally and were in fact specially designed for the *Pelargonium* leaf. Both the upper and lower epidermis is hairy, especially the lower which also has far more stomata than the upper; the variation in stomatal numbers is discussed below (p. 478). The structure of the lower stomata is shown in Fig. 1 in which the average dimensions have been inserted. The full depth of the stomatal pore is about 12μ , but owing to the more or less funnel-shaped entrances to the pore the depth of the narrow part is only about 5μ in the centre of the elliptical opening. The long and short axes of the almost elliptical aperture are least in the midplane of the epidermis, and in this position their values vary from 10 to 20μ for the long axis and from 0 to 10μ for the short axis. These values for the two axes are

¹ The author is indebted to Mr. P. S. Nutman, who very kindly embedded, cut, stained, and mounted the material used in obtaining these data.

obtained from measurements on strips of epidermis taken by Lloyd's method and their validity will be discussed in Part 3. On the outside of the stoma is a very delicate rim of cuticle which somewhat constricts the aperture at this point and appears in section as a pair of 'horns' (Fig. 1). The upper stomata are similar, but tend to be slightly larger.

Begonia sanguineum is also very suitable for the leaf chambers, which are



FIGS. 1 a-c. Lower stomata of *Pelargonium zonale*. The dimensions refer to average values. Fig. 1 a. Camera lucida drawing of transverse section through two open stomata, near centre of aperture. Fig. 1 b. Similar drawing near one end of the elliptical aperture. Fig. 1 c. Diagram of surface view of one of the stomata.

attached to the wider side of the asymmetrical leaf. Since the leaf is glabrous and very smooth it is easy to avoid air leaks into the chambers. Under each epidermis of the leaf is a thick layer of large-celled 'water-storage' tissue. Between these two layers is a palisade about 36μ in thickness and a layer of spongy mesophyll of about 50μ (Fig. 2). Owing to the tapering form of the palisade cells, the spaces in the lower part of this tissue must be available for lateral flow of gas. Stomata, which are present in the lower epidermis only, are grouped together in circular patches of four to ten each (Fig. 3). Abutting on each stomatal patch is a cylindrical tunnel passing through the water storage tissue to the spongy mesophyll (Fig. 2). Gaseous diffusion has therefore to traverse these tunnels which are about 180μ long and 200μ wide; and in the case of porometer experiments air or gas has to pass down one set of tunnels, along through the mesophyll and up a second set. Owing to the great width of the tunnels their resistance is likely to be low, but since the spongy

mesophyll layer is narrow and small veins and cystoliths appear to be numerous the leaf's total internal resistance both to diffusion and viscous flow

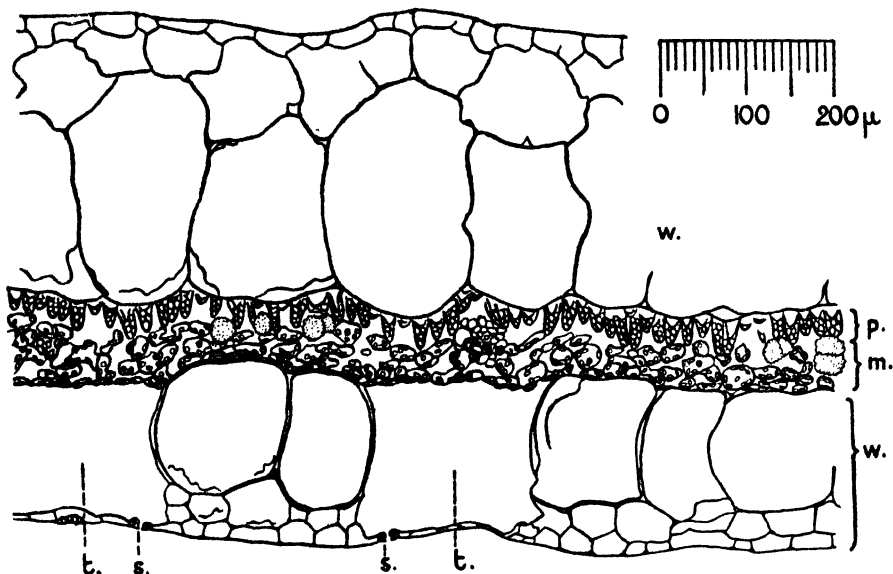


FIG. 2. Transverse section of leaf of *Begonia sanguineum*. *w*, water storage tissue. *p*, palisade parenchyma. *m*, spongy mesophyll. *s*, stoma. *t*, cylindrical tunnel abutting on stomatal group.

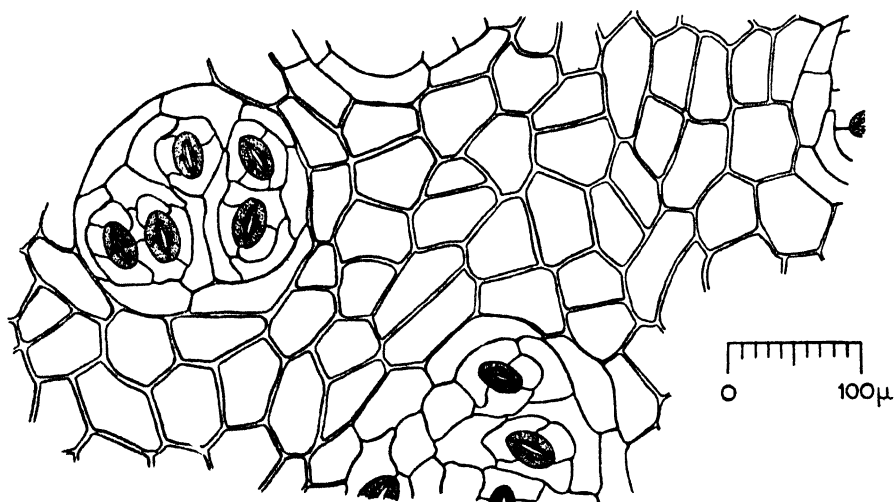


FIG. 3. Lower epidermis of leaf of *B. sanguineum*, showing groups of stomata.

may be expected to be higher than in *Pelargonium*. The stomata in any one patch are about $30\text{--}80\ \mu$ apart and the long axis of the elliptical opening varies between about 12 and $18\ \mu$. No measurements of the short axis have been

made. The total depth of the pore is about $10\ \mu$, but as in the case of *Pelargonium* the depth of the narrow part is less and approximates to $4\ \mu$. The *Begonia* stoma also has a cuticular rim giving the effect of 'horns' in transverse section.

THE RESISTANCE POROMETER

1. Theoretical Considerations

The resistance porometer has been fully described by its originators (Gregory and Pearse, 1934), but a diagram of the form used in the present investigations (Fig. 4) will clarify the following discussion. Air is drawn through the leaf R_2 into a porometer cup by means of a constant pressure aspirator P , one of two alternative standard capillary resistances at R_1 being placed in series with the leaf resistance R_2 , i.e. between the cup and aspirator. A water manometer p_2 measures the pressure drop $(P_1 - P_2)$ across the leaf R_2 and a second manometer p_1 measures the total pressure drop $(P_1 - P_3)$ across $R_2 + R_1$.¹ By difference the pressure drop across the series resistance R_1 is $P_2 - P_3$. It will be noted that the actual measurements made are in terms of pressure differences. These may be used for calculating either the resistance or the conductance of the portion of the leaf through which air flows.

(a) *The resistance formula.* The usual formula, as given by Gregory and Pearse, assumes that the volume V of air flowing through a constant resistance in unit time is proportional to the pressure difference. Thus

$$V = \frac{(P_1 - P_2)}{R_2} k_2 = \frac{(P_1 - P_3)}{R_1 + R_2} k_3 = \frac{(P_2 - P_3)}{R_1} k_1, \quad (i)$$

where V is measured at constant pressure. This is made clear from the geometry of the diagram (Fig. 5). Hence assuming that k has a constant value

$$R_2 = \frac{R_1(P_1 - P_2)}{(P_1 - P_3) - (P_1 - P_2)} = \frac{R_1(P_1 - P_2)}{(P_2 - P_3)}. \quad (ii)$$

Actually the general expression for gaseous flow through a capillary involves the difference of the squares of the pressures at each end, but except when the pressure drop is large the error involved in the simpler assumption is negligible. The formula found for R_2 , assuming that flow through both the leaf and the series resistance is proportional to the difference of the squares of the pressures, is

$$R_2 = \frac{R_1(P_1 - P_2)(P_1 + P_2)}{(P_2 - P_3)(P_2 + P_3)}. \quad (iii)$$

Since $(P_1 - P_3)$ never exceeds 13.6 cm. of water in the present investigations the error in taking $(P_1 + P_2)$ as equal to $(P_2 + P_3)$ does not amount to more than

¹ As mentioned in the previous paper, the necessity for reading p_1 lies in the fact that at high rates of flow the suction produced by the aspirator falls appreciably owing to the resistance to outflow of water.

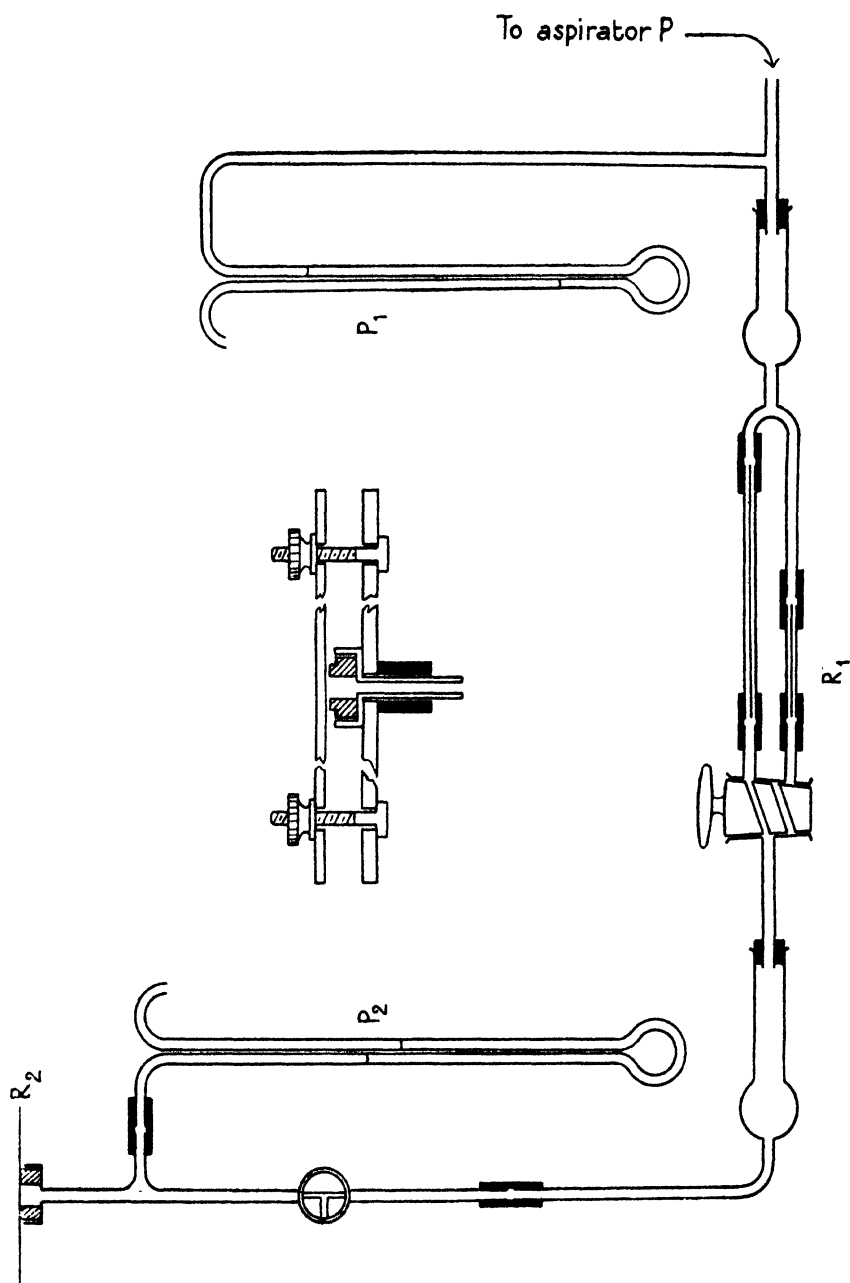


FIG. 4. Diagram of the resistance porometer employed. The enlarged inset shows the method of supporting the small circular porometer cup against leaf. For explanation of figure see text.

about 0.7 per cent. Equation (iii) then reduces to equation (ii). If very large heads are used in the constant pressure aspirator, especially when extreme accuracy is required, it may be better to use the more cumbersome equation (iii), necessitating the use of the absolute pressures.

(b) *The unit of resistance.* It has been assumed above that k has the same

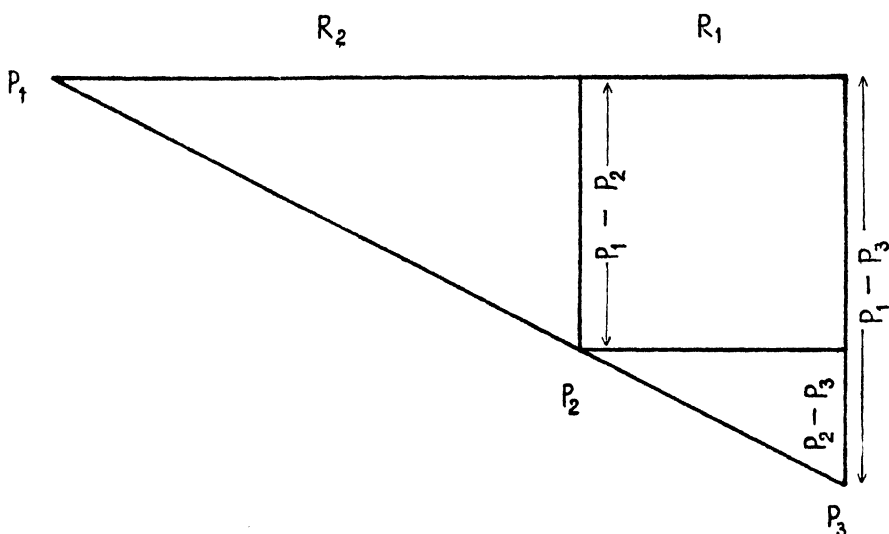


FIG. 5. Diagram illustrating the pressure relations of the resistance porometer.

value for the leaf as for the capillary tube R_1 . In the present work this follows from the definition of resistance used, which may be stated thus: the resistance R of a portion of leaf or a capillary tube is said to be the dimensional value $l/r^4 \text{ cm.}^{-3}$ of an equivalent long capillary tube of circular cross section, having a length l and radius r , which would pass the same flow V of the same fluid for the same pressure drop, i.e.

$$R = \frac{l}{r^4} = \frac{(P_1 - P_2)}{V} \frac{\pi}{8\eta}. \quad (\text{iv})$$

R is thus a dimensional constant obtained by assuming only that flow is proportional to pressure drop, and by definition k_1 , k_2 , and k_3 in equation (i) have all been made equal to $\pi/8\eta$, η being the coefficient of viscosity.

The leaf resistance R_2 is thus found in terms of the capillary resistance R_1 defined as above.

For convenience, arbitrary capillary units are used, except where otherwise stated, such that one unit = $3.77 \times 10^8 \text{ cm.}^{-3}$ (Heath, 1939); this being approximately the same as the unit used by Gregory and Pearse (1934).

The values of R_2 are dimensional, but it does not necessarily follow that the term r^4/l in Poiseuille's formula, or the equivalent expression $2a^3b^3/(a^2 + b^2)l$ for an elliptical capillary tube of semi-axes a and b , is correct in terms of the

actual stomatal dimensions, although it is satisfactory as a value for the conductance of a capillary tube equivalent to the leaf.

(c) *The validity of the resistance determinations.* The assumption that flow through both the leaf R_2 and the series resistance R_1 is proportional to the pressure drops across them, or alternatively to the differences of the squares of the pressures, is vital to the use of the resistance porometer; for if it is appreciably incorrect equations (ii) or (iii) may no longer hold and R_2 may be wrongly estimated. Proportionality between flow and pressure drop, indicating that the former is predominantly viscous in character, may be tested by direct experiment. Such tests were carried out (Heath, 1939, p. 490) for the capillary resistances A and B used at R_1 in these investigations, and flow was found to be proportional to pressure difference up to rates greatly in excess of those used in experiments with leaves. Similar tests may be carried out for flow through a leaf attached to the resistance porometer by taking alternate readings with the two standard resistances. Since one of these (A) has approximately a quarter of the resistance of the other (B), it gives a much larger pressure drop ($P_1 - P_2$) across the leaf. If the same value for R_2 is found with A instead of B at R_1 the rate of flow has increased proportionately with ($P_1 - P_2$). The results of such experimental tests (see section 2 (c) below) give no evidence of non-viscous flow through the leaf. Unfortunately these experiments could not be satisfactorily carried out at the smallest stomatal apertures, although they cover the major part of the range of leaf resistance usually encountered. With very high leaf resistances the use of the lower resistance A at R_1 gives very poor sensitivity owing to the great difference between the values of R_1 and R_2 (see p. 467 below). At the highest leaf resistances, although the narrowness of the stomatal apertures increases the likelihood of turbulent flow, the very greatly reduced rate of flow acts in the opposite direction. Actually Reynold's number is estimated as of the order of unity.¹ It may be noted that Maskell (1928) working with cherry laurel found flow to be proportional to pressure drop with a Darwin type porometer.

It has been shown that from resistance porometer readings valid estimates of leaf resistance may be made on the apparently justifiable assumption of proportionality between flow and pressure difference. It is of course equally legitimate to use the resistance porometer readings for calculating the conductance $1/R_2$, of the leaf. In either case the measure obtained is independent of the pressure drop employed and is in this respect preferable to measurements of flow as obtained with the Darwin and Pertz (1911) or the Knight (1915) porometer in that it more nearly approaches to a measure characteristic of the leaf dimensions only. Values obtained with a flow type of porometer, if calibrated in actual rates of flow, may however be used to calculate the resistance or conductance from equation (iv).

¹ Reynold's number = $vd\rho/\eta$, where v = velocity of flow; d = diameter of tube; ρ = density of fluid; η = viscosity of fluid. The critical value above which turbulent flow may be expected to appear is approximately 2,000.

Over the range of leaf resistance covered by the experiments described in section 2 (c) and mentioned above the proportionality found between flow and pressure drop disposes of the criticism (cf. Darwin, 1916; Knight, 1916; and Stålfelt, 1929) that the flow of air through the stomata may force them open to an appreciable extent.

(d) *Size of cup and sensitivity.* The use of a small cup for calibrations is made necessary by practical considerations, such as the need for many positions on the same leaf for calibration in terms of stomatal aperture, and the desirability of keeping the area, and hence the diffusion rate, reasonably small for calibration in terms of diffusive resistance.¹ For the actual assimilation experiments a large porometer cup, such as the leaf chamber *LI*, Heath (1939), is advantageous because, with the relatively low values of R_2 that it gives, equilibrium is more rapidly attained. Furthermore, the manometer reading (p_2) oscillates more rapidly and with less amplitude with the bubbling in the aspirator when R_2 is low, and therefore the mean reading may be obtained with greater speed and accuracy. The actual shape of *LI* was of course dictated by the shape of the *Pelargonium* leaf and the necessity for avoiding the thicker parts of the veins, yet having a sufficiently large area for the assimilation measurements.

In relation to sensitivity it seems to be a matter of indifference whether a large or small cup is used, provided that a suitable resistance is chosen for R_1 . Gregory and Pearse (1934) showed that for given values of $(P_1 - P_3)$ and R_1 the sensitivity of the resistance porometer, i.e. the change in $(P_1 - P_2)$ for unit change of R_2 , was greatest when R_2 was minimal, and this would appear to favour a large cup. On the other hand, maximum sensitivity in terms of conductance $1/R_2$, i.e. the greatest change in $(P_1 - P_2)$ for unit change of $1/R_2$, is obtained when R_2 is maximal which seems to favour a small cup. If use is made of $\log R_2$ or $\log 1/R_2$, the change in $(P_1 - P_2)$ for unit change of $\log R_2$ is the same as that for unit change of $\log 1/R_2$. Moreover this sensitivity would be constant for all values of R_2 , if R_1 could be maintained almost equal to R_2 . Gregory and Pearse (1934) give the equation for sensitivity in terms of R_2 , which in our notation is:

$$\frac{d(P_1 - P_2)}{dR_2} = \frac{(P_1 - P_3)R_1}{(R_1 + R_2)^2},$$

where R_1 and $(P_1 - P_3)$ are maintained constant. From this it may be shown that the sensitivity in terms of $\log_e R_2$ is:

$$\frac{d(P_1 - P_2)}{d \log_e R_2} = \frac{(P_1 - P_3)R_1}{(R_1 + R_2)^2} R_2.$$

Hence, if R_1 is made equal to R_2 ,

$$\frac{d(P_1 - P_2)}{d \log_e R_2} = \frac{(P_1 - P_3)}{4}.$$

¹ With *Begonia*, in which both the flow and diffusive resistances are much higher than in *Pelargonium*, a rectangular cup 8×1 cm. is used for calibrations instead of a small one.

If, therefore, R_1 can be maintained approximately equal to R_2 , the sensitivity in terms of $\log_e R_2$ (or $\log_e 1/R_2$) is constant at a value equal to $1/4$ of the pressure drop across the whole system. The corresponding sensitivity in terms of $\log_{10} R_2$ is of course $2.303(P_1 - P_3)/4$.

When $R_1 = R_2$, sensitivity is maximal not only in terms of R_2 , as was shown by Gregory and Pearse, but also in terms of $\log R_2$ or $\log 1/R_2$. This may be shown by differentiating $(P_1 - P_2)$ a second time with respect to $\log_e R_2$:

$$\frac{d^2(P_1 - P_2)}{d(\log_e R_2)^2} = \frac{(P_1 - P_3)R_1 R_2}{(R_1 + R_2)^2} \left\{ 1 - \frac{2R_2}{(R_1 + R_2)} \right\},$$

where R_1 and $(P_1 - P_3)$ are maintained constant. Equating this to zero gives maximum sensitivity when $R_1 = R_2$.

If R_1 is of the same order as R_2 , the change in sensitivity in terms of $\log R_2$ or $\log 1/R_2$ is found to be small over a large range of R_2 , e.g. if $(P_1 - P_3)$ is maintained constant at 10 units and R_2 is increased or decreased five-fold above or below R_1 , giving a total range of twenty-five times, the sensitivity in terms of $\log_e R_2$ falls off symmetrically on either side of the maximum value of 2.50 to 1.39 at the extremes of the 25-fold range of R_2 . On the other hand, for a 25-fold increase in R_2 the sensitivity in terms of R_2 decreases 7.4 times when, in order to secure throughout the best sensitivity, R_1 is made equal to the mean of the extreme values of R_2 . When $R_1 = R_2$, the sensitivity in terms of R_2 is $(P_1 - P_3)/4R$ and thus depends on the value of R_2 , unlike that in terms of $\log R_2$ given above. This relatively small variation in sensitivity in terms of logarithmic values makes it desirable from a statistical point of view to use such values whenever possible in order to maintain the errors as constant as may be, since the error of reading the manometer p_2 is approximately the same throughout the whole range.¹ It is also of course desirable to maintain R_1 as similar to R_2 as is practicable, and a glance at the level of manometer p_2 will show when this is achieved, since $(P_1 - P_2) = \frac{1}{2}(P_1 - P_3)$ when $R_1 = R_2$.

The above considerations with regard to sensitivity of course apply to the total resistance R_2 or conductance $1/R_2$ as measured. When calculated values of $1/s_1$ or s_1 (see next section) are used, the results given above will be modified especially at large stomatal apertures where the correction required for the effect of the resistance of the intercellular spaces and the stomata outside the cup is great.

(e) *The relation between stomatal resistance and total resistance measured with a circular porometer cup.* All porometer methods are open to the criticism that the readings are affected not only by the resistance of the stomata within the cup but also by the resistance of the intercellular spaces and the stomata outside the cup. The combined resistance of the stomata outside the cup would be negligible, since their number is usually large compared with the number

¹ Actually the manometer readings are slightly more accurate at lower values of $(P_1 - P_2)$ owing to the smaller and more rapid oscillation mentioned earlier.

within the cup, were it not for the effect of resistance to flow in the intercellular spaces. The existence of the latter, which for convenience may be termed 'mesophyll resistance' since nearly all lateral flow must take place through the spaces in the spongy mesophyll, adds greatly to the complexity of the problem.

Stålfelt (1929) considered that the presence of the mesophyll resistance and the existence of other sources of error rendered impossible the finding of a relation between air flow and stomatal aperture. Darwin (1916) concluded that the stomata within the cup provided the limiting control of air flow since cutting the leaf near the cup did not seem to increase the rate. He appears to have overlooked the possibility of infiltration of the intercellular spaces by sap from the injured cells and of 'shock' closure of the stomata within the cup.

Knight (1916) measured the reduction of flow caused by blocking the stomata over a known area surrounding the porometer cup, using either vaseline or his ingenious double cup. In this way he estimated the effect of the mesophyll resistance for an extra length of path through the leaf. He concluded that this resistance was considerable and might markedly affect porometer readings, and he considered that since it was approximately constant its relative importance increased as the stomata opened. Newton (1936) drew attention to the following important consideration: '... as the stomata open the flow through those remote from the cup will be less, and hence less mesophyll will be included in the path of flow.' He made the first attempt known to the author to partition the various measured total resistances R into component resistances attributable to the stomata within the cup R_i , to those immediately above it R_u , and to the intercellular spaces plus other stomata outside the cup R_m . For this purpose he made use of an electrical analogy, devised by Mr. Baggally, in which the leaf was considered as a system of three plates pressed together. The two outer plates representing upper and lower epidermis respectively conducted only at right angles to their plane surface with conductances in the ratio of the numbers of stomata per unit area. The middle sheet representing the other leaf tissues ('mesophyll') was assumed to be without resistance at right angles to the leaf surface, being very thin compared with the radius of the leaf, but to have a superficial resistivity m . Current was supposed to flow from a circular central electrode representing the porometer cup on the lower surface of the leaf, through the stomata inside the cup, along the mesophyll, and to leak away through all the remaining stomata to an earthed conducting fluid bathing the leaf outside the cup. The system was split into two parts, that opposite the cup where the current passed directly through the leaf and that beyond the cup where the current passed along the mesophyll and leaked away through the remaining stomata.

Newton estimated m , the superficial resistivity of the mesophyll, by means of double cup experiments similar to those of Knight (1916), but he seems to have overlooked the fact that this should have been done at the widest possible stomatal apertures so that with the outer cup open the minimum of air would be

flowing through the mesophyll.¹ Actually all his three double-cup experiments were carried out at medium stomatal apertures and all gave almost the same resistance for the extra area of mesophyll. His value of m must therefore have been an underestimate. He assumed a ratio of lower to upper stomata of 4 to 1, and for various assumed values of stomatal conductance s (where $s = 4s/5 + s/5$) he calculated R_l , R_u , and R_m . Hence he obtained the total resistance R from

$$R = R_l + \frac{R_u R_m}{R_u + R_m}.$$

He found that over most of the very great range of R that he explored experimentally R_l accounted for almost the whole resistance, but at maximal stomatal openings $R_u R_m / (R_u + R_m)$ became important.

He also calculated from his electrical analogy the percentages of the total air current which passed straight through the leaf or across rings of stated radius round the cup. Owing to relative changes in R and R_m the distribution of the air stream was found to differ greatly at large and at small stomatal openings. Thus for a cup of radius 0.5 cm. at the smallest apertures 0.21 per cent. passed straight through above the cup and 97 per cent., 85 per cent., and 36 per cent. across rings of radius 1, 2, and 4 cm. respectively. At the widest aperture on the other hand 14 per cent. of the air passed straight through and 15 per cent., 0.38 per cent., and 0.0001 per cent. across the above-mentioned rings.

The treatment used by Newton appears open to two main criticisms. The first is that no account is taken of the resistance to lateral flow in the mesophyll above the cup, since he assumes in the calculations that current flows directly through the leaf to the upper stomata above the cup or else laterally from the edges of the cup. Actually there would also be some lateral flow above the cup. The second criticism is that no account is taken of the effect of the width of the washer attaching the porometer cup to the leaf. To complete the analogy this should be represented by a ring-shaped insulator surrounding the central electrode. A third criticism is to be levelled at Newton's apparent assumption that for the purposes of the analogy the radius of the *Pelargonium* leaf might be taken as 10.0 cm. (Newton, 1936, p. 23). For a circular porometer cup of 0.5 cm. radius the mean radius of surrounding leaf tissue unobstructed by main veins would be nearer 2.0 cm., and certainly not more than 3.0 cm. even for the largest leaf. This might be expected to modify considerably the calculated values of R_m and the theoretical distribution of flow in the different parts of the leaf.

Dr. H. L. Penman has very kindly made a new mathematical investigation of the problems of distribution of air flow in a *Pelargonium* leaf with a porometer cup attached. The problem is treated on lines somewhat similar to that of the electrical analogy used by Newton, but allowance is made for the

¹ Newton calculated $m = \frac{2\pi(R_3 - R_2)}{\log_e a_3/a_2}$ with no further correction (cf. Appendix, p. 495).

effects of lateral flow above the cup and of the width of the washer. In this investigation the following assumptions have been made:

1. That the rate of air flow varies directly as the pressure difference. This seems justifiable since the pressure differences are small and lack of turbulence has been shown experimentally over a considerable range of stomatal opening (see section 2 (c) below).

2. That flow at the boundary, a distance b from the centre of the cup, is zero. The value chosen for b is the approximate mean distance of the edge of the leaf and of main veins.

3. The conductances s_2 and s_1 of the upper and lower epidermis respectively are assumed to be in the ratio of the numbers of stomata. This implies negligible interference between the stream lines flowing through neighbouring stomata and also that the mean stomatal apertures are at all times the same on both surfaces. Newton (1936) found that the stomata on the two surfaces opened and closed synchronously. The upper stomata have a tendency to be somewhat larger than the lower, when examined by Lloyd's method, but against this may perhaps be offset the resistance to flow in the spaces of the palisade parenchyma (see 4 below, but also section 2 (d) and Discussion).

4. The intercellular space resistance is assumed to be negligible in a direction at right angles to the plane of the leaf. This seems reasonable in view of the shortness of the path and the fact that intercellular spaces predominantly run in this direction. Such resistance as occurs in the palisade tissue will be additional to that of the upper stomata, and hence at large apertures there may be a tendency for s_2/s_1 to fall (but see section 2 (d) and Discussion).

5. Since it is assumed that the mesophyll has a resistance to flow only in directions parallel to the leaf surface (assumption 4) its 'specific resistance' m may be termed a surface resistivity. The value of m is assumed to be constant which implies no change in volume or shape of the intercellular spaces with stomatal movement. This seems likely to be a reasonable approximation as long as the leaf is not allowed to wilt,¹ and some experimental evidence for the constancy of m has been obtained (see section 2 (d) below).

The mathematical argument is presented by Dr. Penman in the Appendix. Briefly, it may be stated here that the system is considered in three parts: the area of leaf of radius a_1 covered by the circular cup, the area covered by the washer of width $a_2 - a_1$, and the remainder of the leaf up to the boundary of radius b . In this outer part of the leaf air flows into an elementary annulus from above, below and the periphery and out of it towards the centre; above the washer air flows in from above and the periphery only and again leaves the annulus towards the centre; while over the cup air flows into such an

¹ Pearse (1935) found that the leaf of *Pelargonium* wilted when the percentage water content of a fully turgid leaf fell by less than 2. The shrinkage resulting from this would have an entirely negligible effect on diffusive resistance and the effect even on flow resistance would not be large.

annulus from above and the periphery, and leaves it towards the centre and towards the cup. At the centre of the cup there is no lateral flow. From a theoretical consideration of these effects a series of equations has been obtained enabling $1/R_2$, the total conductance, to be calculated from the following physical characteristics of the system:

- a_1 the radius to the inside of the washer,
- a_2 the radius to the outside of the washer,
- b the radius to the boundary,
- m the surface resistivity of the mesophyll,
- s_1 the conductance of the lower epidermis,
- s_2 the conductance of the upper epidermis,

and from derived functions β_1 and β_2 which equal $\sqrt{m(s_1+s_2)}$ and $\sqrt{ms_1}$ respectively. The only unknowns are s_1 and s_2 , which have a known ratio α , and m which can be evaluated by experiment. Modified equations for a hypostomatous leaf ($s_2 = 0$) have also been derived. By the use of these equations, values of m/R_2 can be calculated for various assumed values of β_1 , and in this way a series of curves relating $\log m/R_2$ to $\log ms_1$ may be constructed for different values of α (the ratio s_2/s_1). From this known ratio and the experimentally determined value of m/R_2 the value of $\log ms_1$ may be read off from the curves, either directly or by interpolation.¹ The calculation enabling the curves to be plotted proceeds by a series of steps using in turn the equations (4), (5) and (6a) given in the Appendix. For the hypostomatous leaf ($\alpha = 0$), the term in the square bracket on the left-hand side of equation (4) [M of equation (7)] and equation (7) only are needed. A sample calculation for an amphistomatous leaf ($\alpha > 0$) will now be presented to demonstrate the method of procedure:

Let $a_1 = 0.45$; $a_2 = 0.70$; $b = 2.0$; $\alpha = 0.04$, and take a value of β_1 such that $\beta_1 a_1 = 0.20$.

With these values the term in the square bracket on the left-hand side of equation (4) of the Appendix (M of equation 7) is found to be -1.073 .

$$\text{Hence } \frac{\beta_2 M}{\beta_1} = \sqrt{\left(\frac{s_2}{s_1+s_2}\right)} M = \sqrt{\left(\frac{\alpha}{1+\alpha}\right)} M = -0.210$$

$$\text{and } F/G = -1.0487.$$

$$\text{Hence right-hand side of (5) } = 0.152 = N.$$

$$\begin{aligned} \text{Then } \frac{m}{R_2} &= \frac{2\pi\beta_1 a_1}{(1+\alpha)^2} \left[\alpha \beta_1 a_1 - \frac{1}{\sqrt{\left(\frac{1+\alpha}{\alpha}\right) N - \frac{I_0(\beta_1 a_1)}{I_1(\beta_1 a_1)}}} \right]. \quad (6a) \\ &= 0.111. \end{aligned}$$

$$\text{Also, } ms_1 = \frac{\beta_1^2}{1+\alpha} = 0.190.$$

¹ Note that R_2 is the total resistance found with the size of cup used and s_1 is the stomatal conductance per unit area of lower epidermis.

A set of three typical curves is shown in Fig. 6. They relate to a circular cup of radius 0.45 cm. having a washer 0.40 cm. wide, with an assumed value for the distance of the boundary b of 2.0 cm. The three curves are for three different ratios (α) of upper to lower stomatal numbers, namely 0.00, 0.04, and 0.10.

In order to use such curves for calculations m must be known. In the present

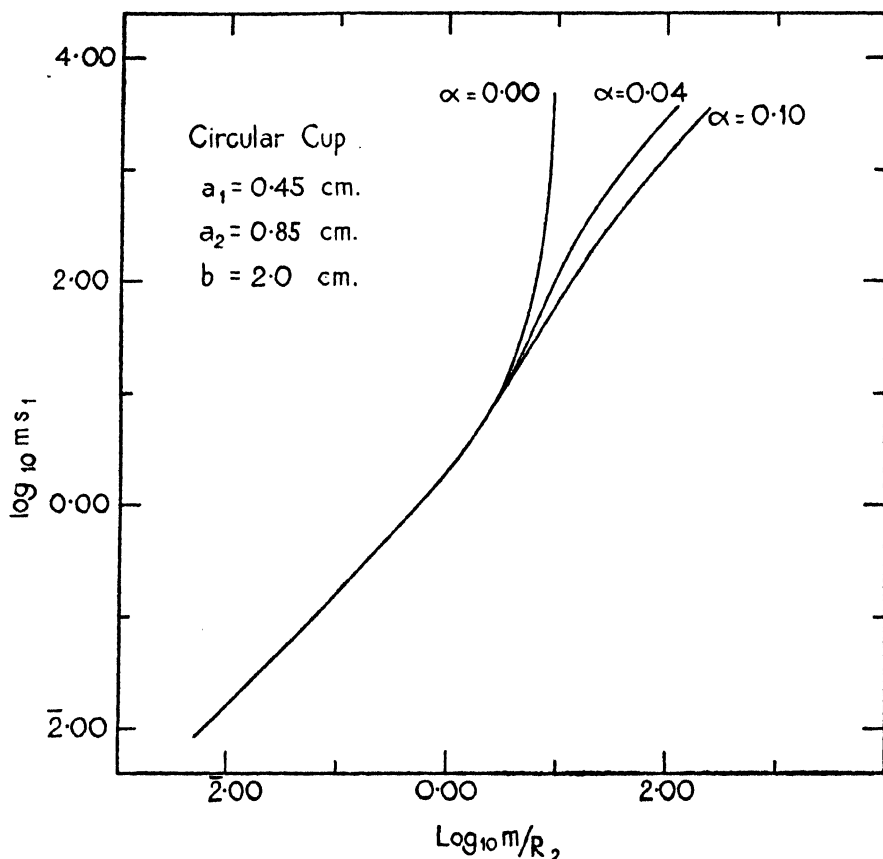


FIG. 6. Curves illustrating theoretical relationship between total resistance R_2 and stomatal conductance per unit area s_1 .

investigations the leaf chambers (Heath, 1939) have been used for double-cup experiments (see section 2 (*d*) below) and m has been estimated by the method of successive approximation given in the second part of the Appendix (p. 497). The value of 4.85 units found for *Pelargonium* is derived from experiments with only two leaves, while that for *Begonia* (16.6) is based on a single leaf. Obviously if a single value of m is to be used for all leaves of the same strain it should be based on much wider experimentation. It would also have been preferable to carry out the double-cup experiments with a circular double cup

similar to Knight's. As mentioned in the Appendix, the theory of flow distribution for a circular cup involves fewer approximations than that for an elongated cup, especially in the case of the outer part of a double cup when a_2 is large. If the inner circular cup were placed symmetrically across a major vein a reasonably large area of leaf unrestricted by veins would be obtained beyond the outer cup. (See Appendix, p. 495, where the method of calculating m from the results of experiments with a circular double cup is described.) It is hoped to carry out such further experimentation at a future date. Meanwhile the approximate values of m obtained will serve to show the types of results that may be expected. It may be mentioned that the two double-cup experiments carried out in May and December respectively with *Pelargonium* leaves of very different size gave very consistent estimates of m .

Using the above-mentioned values of m the extreme range of $\log ms_1$ in the author's experiments is from $\bar{2}\cdot4$ to $3\cdot3$, this being for *Pelargonium*. These figures show that practically the whole of the range plotted in Fig. 6 may be needed, although of course that usually encountered is much smaller, say $0\cdot0$ to $2\cdot5$ for *Pelargonium* and $1\cdot5$ to $1\cdot0$ for *Begonia*. Dr. Penman has pointed out (Appendix, p. 499) that the value of m becomes extremely critical when the slope of the curve exceeds 2, and it is worthy of note that if α is not less than $0\cdot04$ the slope scarcely exceeds 2 on any part of the curves for the cups used, whether circular or elongated. For a hypostomatous leaf such as *Begonia* ($\alpha = 0$) the slope of the curve does not reach 2 until $\log ms_1$ reaches a value of at least $0\cdot8$.

It may be useful to tabulate for *Pelargonium* some typical calculated values of R_2 and $1/s$ falling within the observed range of $\log ms_1$, in order to demonstrate the importance of correcting R_2 for the effects of the mesophyll and outer stomatal resistances. Since $1/s$ represents the stomatal resistance within the cup and R_2 the total resistance the difference between these values is a measure of mesophyll plus outer stomatal resistance. (See Table I.)

TABLE I

Pelargonium

Values of Total Resistance, R_2 , with a circular Cup of $0\cdot636 \text{ cm.}^2$ area corresponding to various stomatal Resistances ($1/s$ per $0\cdot636 \text{ cm.}^2$ of lower epidermis)

$$a_1 = 0\cdot45; a_2 = 0\cdot85; b = 2\cdot0; m = 4\cdot85$$

$\log ms_1$	$1/s$	R_2		
		$\alpha = 0\cdot0$	$\alpha = 0\cdot04$	$\alpha = 0\cdot10$
$\bar{2}\cdot4$	307	331	331	331
$1\cdot0$	76·9	83·3	83·3	83·3
$0\cdot0$	7·69	8·93	8·93	8·93
$1\cdot0$	0·769	1·70	1·62	1·51
$1\cdot5$	0·243	1·05	0·870	0·775
$2\cdot0$	0·0769	0·775	0·513	0·380
$2\cdot5$	0·0243	0·662	0·282	0·166
$3\cdot0$	0·00769	0·588	0·123	0·0645
$3\cdot3$	0·00386	0·562	0·0690	0·0347

For the two smallest values of $\log ms_1$ tabulated, the difference between $1/s$ and R_2 , amounting only to 7–7.5 per cent., is attributable almost entirely to the finite value of b , i.e. to the limited number of stomata outside the cup. This is shown by the fact that the ratio, 0.94, of the reciprocal of the area inside the cup to the sum of the reciprocals of the areas inside and outside is almost the same as the ratio of $1/s$ to R_2 , namely 0.93. As the stomata open, the importance of b rapidly diminishes and hence even large errors in its value are relatively unimportant as long as $b^2 - a_2^2$ is large compared with a_2^2 . On the other hand the effect of the upper stomata increases rapidly in importance with increasing stomatal conductance. This can be clearly seen both from the curves in Fig. 6 and the values in Table I. It is evident that for the dimensions under discussion and with a hypostomatous leaf ($\alpha = 0$), values of R_2 are a very poor measure of stomatal resistance when $\log ms_1$ exceeds 2.0. This is because R_2 is tending to a constant value, 0.492, given by equation (10) of the Appendix and due entirely to the resistance of the mesophyll above the washer. Under these circumstances not only will the value of m be very critical, but any errors in measuring the width of the washer will have a disproportionately large effect on the value of $1/s$ obtained. It is evident that porometer determinations without correction for mesophyll resistance will lead to greater errors in the case of hypostomatous than amphistomatous leaves, as has been pointed out by Stålfelt (1929) and Newton (1936), though even in the latter case these errors may be considerable at wide or medium apertures unless α is very large.

The greater the value of α , the more nearly does the curve relating $\log ms_1$ to $\log m/R_2$ approach to a straight line and hence the smaller the errors in uncorrected resistances.

Some of these consequences of the theoretical treatment are illustrated, from actual experimental data, by Fig. 7 which shows time curves for logarithms of total resistance R_2 and stomatal resistance $1/s$ for the same cup area during stomatal opening and closure. Values of $\log ms_1$ are also plotted to indicate the position on the theoretical curves of Fig. 6. The curves relate to two experiments, one (28.11.39) with a hypostomatous and the other (16.11.39.) with an amphistomatous *Pelargonium* leaf; in the latter the ratio of upper to lower stomata was 0.10. The dimensions of the cups were the same as in Table I. The curves clearly show (1) that at high resistances $\log 1/s$ differs by an almost constant amount from $\log R_2$, this being almost entirely an effect of b , (2) that at low resistances with the hypostomatous leaf very small changes in $\log R_2$ correspond to large changes in $\log 1/s$, whereas with the amphistomatous leaf R_2 provides a more sensitive measure of $1/s$ although here also the difference between $\log R_2$ and $\log 1/s$ is considerable.

(f) *The relation between stomatal resistance and total resistance measured with an elongated porometer cup.* The Appendix also includes an approximate theory of distribution of air flow in a leaf with an elongated porometer cup, such as the leaf chamber LI (Heath, 1939) or the rectangular cup 8×1 cm.,

used for certain experiments with *Begonia*. Here it has been found necessary to make the simplifying assumptions that the cup is a rectangle of length l

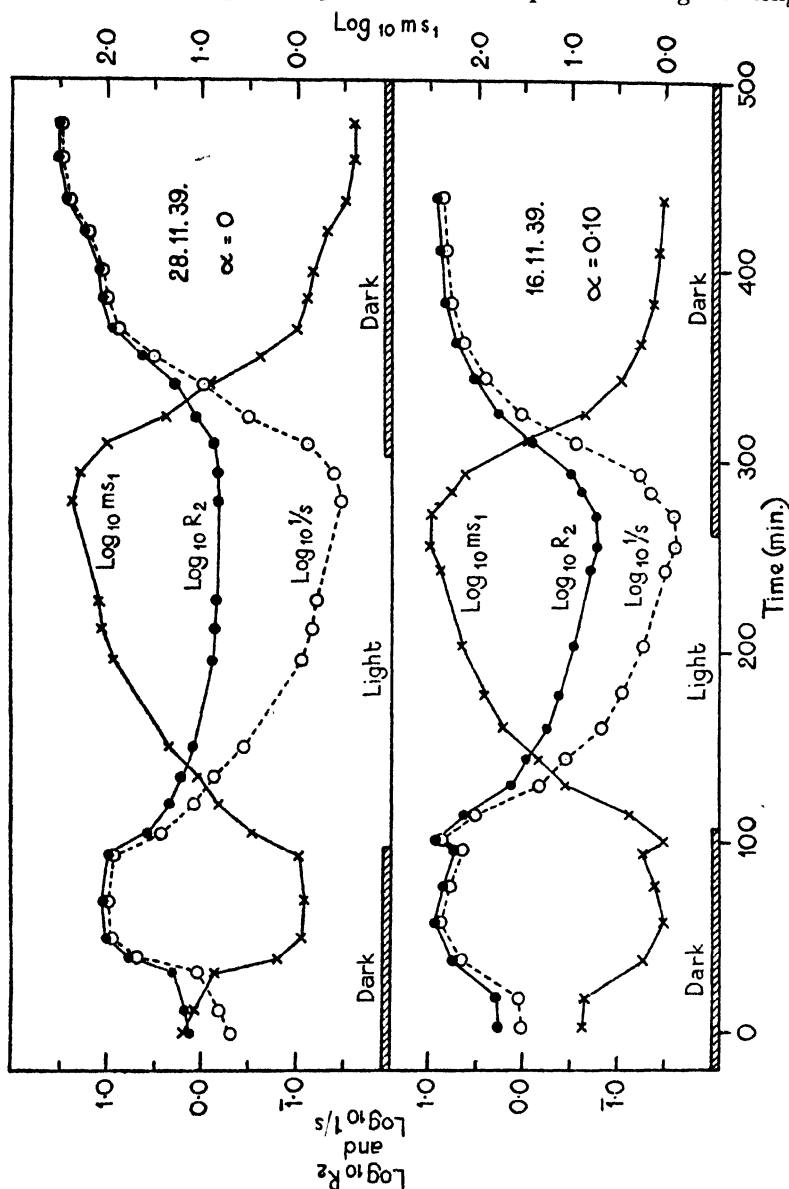


FIG. 7. *Pelargonium*. Variation in logarithms of total resistance R_2 , stomatal resistance within the cup $1/s$, and ms_1 during stomatal opening and closure.

(equal to the area divided by the width $2a_1$) and that the effects of flow across the ends of the cup are negligible. The effects of the upper washers of the leaf chambers and of that surrounding the lower outer chamber LO have also been

neglected, while the other assumptions remain the same as previously stated for a circular cup. The value chosen for b is the approximate mean distance from the centre line of the cup to the edge of the leaf on one side and the main veins on the other. a_1 and a_2 are the distances from the centre line to the inside and outside respectively of the washer surrounding LI . From the equations (12) and (13) of the Appendix values of m/R_2 corresponding to assumed values of β_1 can be calculated. Curves relating $\log m/R_2$ to $\log ms_1$ can be plotted for different values of α . These curves resemble those for a circular cup and similar considerations apply to them. At small values of $\log ms_1$ b is more important than for a circular cup of the same a_1 and a_2 at the same value of b , but again the effect of b disappears rapidly as the stomata open. The neglect of the effect of the washer surrounding LO will also be of most importance at small stomatal apertures. For the dimensions used, the effect of the upper stomata is apparent in the separation of the curves at a lower value of $\log ms_1$ than for a circular cup.

Since the theory for the leaf chamber is much less precise than that for a circular cup, experimental tests of its validity are especially desirable. Three types of experiment which may give information as to the adequacy of the theory have been carried out:

First, the lower leaf chambers have been used as a double cup for the estimation of m with the upper chambers closed so as to simulate hypostomatous conditions. If, in such experiments, the value of m obtained by the method of successive approximation (Appendix, p. 497) is found to be nearly constant over a considerable range of stomatal conductance, this may be taken as evidence both for the constancy of m and for the correctness of the *shape* of the $\alpha = 0$ curve over the range studied. It should be pointed out that any error acting as a multiplying constant, e.g. the effective value of l being different from that actually used, will not affect the constancy but only the value of m found as above. Such an error will merely displace the curve relating $\log m/R_2$ to $\log ms_1$ without altering its shape.

Secondly, experiments have been carried out with the leaf chambers in which comparisons between the resistances R_h under hypostomatous and those R_a under amphistomatous conditions have been made by closing or opening the upper chambers. Hence curves relating $\log R_h/R_a$ to $\log m/R_a$ may be drawn for the appropriate values of α . Similar curves may be derived from the theoretical $\log m/R_2$ v. $\log ms_1$ curves by taking the differences $\log m/R_a$ minus $\log m/R_h$ (i.e. $\log R_h/R_a$) at given values of $\log ms_1$; these differences being plotted against $\log m/R_a$. The observed and theoretical curves should agree.

Both the above tests might be made for a circular cup also, given the necessary apparatus, and it is hoped to carry out the first of them at some future date.

Thirdly, comparisons have been made between stomatal conductance derived from the total resistance as measured with the leaf chamber LI on

one part of a leaf and that obtained at the same time with a circular cup on another part of the same leaf. If it is assumed that the theory for the circular cup is adequate and that the stomata open to the same extent in different parts of the leaf agreement between the values obtained over a wide range of stomatal conductance will provide evidence of the validity of the theory for an elongated cup.

As described in the previous paper, when the leaf chambers are used for assimilation experiments in which air is drawn *through* the leaf tissues it is necessary to trim off the parts of the leaf outside the outer chambers and grease the cut edges in order to prevent errors due to lateral diffusion of carbon dioxide. This reduces b to a very low value (1.1 cm.), but although the effect of the resistance of the stomata outside the cup is thereby greatly increased there is the advantage that the value of b is known with some accuracy. Unfortunately, during an 'over' experiment which is to be followed by a 'through' experiment it is necessary to have the knife in position round the part of the outer chambers nearest the edge of the leaf. This restricts the area available on that side, for inflow of air, to the outer chamber ($b = 1.1$), although on the petiole side of the chambers there is no such restriction ($b = 3.0$ say). A mean value of b , generally 2.0, is therefore used as an approximation in such experiments. In order to test this approximation comparisons have been made of the results obtained with the leaf chamber with the knife in position and those obtained with a small cup on the same leaf.

2. Experimental

(a) *Apparatus.* Up to the summer of 1939 the resistance porometer apparatus used was that described in the previous paper, except that for some purposes the leaf chamber *LI* was replaced by a small porometer cup. This was a circular brass cup supported in an ebonite frame and holding a gelatine washer about 0.25 cm. wide and of about 0.45 cm. inside radius (a_1). The surface of the washer was greased as described for the leaf chambers in the previous paper and the leaf was pressed lightly against it by means of a glass or perspex plate held by two screws in the ebonite frame (Fig. 4). For the comparison of the resistance and diffusion porometers special cups were used which will be described in the appropriate context in Part 2 of this series. In these the internal radius a_1 of the gelatine washer was again 0.45 cm., but its width was 0.40 cm. After September 1939 porometer experiments were carried out in a field laboratory belonging to the Imperial College Laboratory at Rothamsted Experimental Station. The same capillary resistances A and B were used, and manometer p_2 was of precision bore tubing and read with a horizontal microscope as before. Manometer p_1 , however, was of ordinary tubing, though selected for even bore, and it was therefore necessary to read both limbs.

It should be mentioned that tests showed the resistance to air flow of the taps and calcium chloride tubes of the apparatus to be negligible.

(b) *Areas and stomatal numbers.* After a porometer experiment the cup is removed and strips of epidermis are taken within and above the cup area, using Lloyd's method, for counts of stomata on the two surfaces. It will be shown in Part 3 of this series that this method gives valid estimates of number of stomata per unit area. The area of leaf covered by the cup is measured. In the case of the small circular cup this is generally effected by measuring several diameters with an accurate scale under a dissecting microscope. For the leaf chamber *LI* the outline of the chamber as shown by the grease is traced through to a sheet of paper by puncturing the leaf with a sharp pencil, and the tracing is afterwards measured with a planimeter. If the cup area differs slightly from that for which a curve of $\log m/R_2$ against $\log ms_1$ is available, interpolation between two curves is carried out. This is also resorted to when a curve is not available for the observed ratio of upper to lower stomata. Values of s_1 (conductance per unit area) have been found and the conductances have also been calculated per 10,000 stomata S_s . The latter measure is preferable for many purposes as there are considerable variations in number of stomata per unit area between leaves and even between different parts of the same leaf. Thus in the *Pelargonium* leaves used the mean number of lower stomata has been found to vary from about 10,000 to 20,000 per cm^2 , and sometimes the number varies by as much as 40 per cent. between two positions on the leaf. The upper stomata are even more variable, ranging in different leaves from 0 to about 1,800 per cm^2 , and their ratio to the lower stomata ranging from zero to 0.18 but being generally less than 0.10. In *Begonia sanguineum* there are no upper stomata and the lower stomata are far less numerous and less variable in number. Thus in the leaves used the number per cm^2 varies between leaves from about 6,300 to 6,800. The maximum variation noted between two positions on the same leaf was 36 per cent.

(c) *Tests of the proportionality of flow and pressure drop.* These tests have been carried out by taking alternate readings with the standard resistances *A* or *B* at R_1 (see p. 465, above) during several experiments and the results are presented in Table II. Owing to changing stomatal aperture it is necessary to interpolate for one or the other sets of data, and interpolated values are shown in italics in the table. Logarithms of R_2 have been used in order to equalize the errors as much as possible (see section 1 (*d*)) and natural logarithms have been preferred in order that the differences (values in column 3 minus values in column 6) may represent a relative change which when multiplied 100 times will give percentage change in R_2 . Values of $\log ms_1$ have been tabulated in ascending order for each experiment and it will be seen that the differences in $\log_e R_2$ (column 3 minus column 6) show no general trend with changing stomatal conductance. It should be noted that positive differences correspond to an apparent increase of resistance with increase of $(P_1 - P_2)$, such as would occur with turbulent flow, and it will be seen that the mean difference is negative. This mean is shown by the *t* test (Fisher, 1925-38)

TABLE II

*Pelargonium**Tests of Proportionality of Flow and Pressure Drop Across the Leaf*

1 unit of resistance = 3.77×10^8 cm.⁻³. Interpolated data in *italics*.
Pressure drop cm. of H₂O.

1. Date of experiment, and cup used.	$R_1 = 0.299$ units.			$R_1 = 1.17$ units.			Dif- ference 3-6.
	2. $P_1 - P_2$	3. $\log_e R_2$	4. $\log_{10} ms_1$	5. $P_1 - P_2$	6. $\log_e R_2$	7. $\log_{10} ms_1$	
3.5.39.	7.40	-0.823	0.44	3.40	-0.741	0.39	-0.082
Leaf chamber	7.16	-0.764	0.41	3.38	-0.753	0.40	-0.011
LI	6.31	-1.051	0.58	2.68	-1.059	0.60	+0.008
(with knife)	3.49	-2.060	1.43	1.12	-2.096	1.46	+0.036
3.5.39.	8.44	-0.275	1.55	4.68	-0.256	1.53	-0.019
Small circular	8.04	-0.436	1.72	4.25	-0.413	1.69	-0.023
cup.	7.93	-0.479	1.78	4.12	-0.466	1.75	-0.013
	7.26	-0.730	2.01	3.48	-0.714	2.00	-0.016
27.4.39.	5.30	-1.400	0.86	1.96	-1.457	0.90	+0.057
Leaf chamber	5.08	-1.475	0.92	1.84	-1.528	0.97	+0.053
LI	3.56	-2.038	1.41	1.34	-1.898	1.29	-0.140
(with knife)	2.78	-2.375	1.68	0.84	-2.410	1.71	+0.035
27.4.39.	7.60	-0.607	1.92	3.86	-0.564	1.86	-0.043
Small circular							
cup.							
24.10.39.	9.00	+0.148	1.19	5.72	+0.122	1.21	+0.026
Small circular							
cup.							
7.10.38.	8.83	-0.178	1.30	5.42	-0.039	1.18	-0.139
Small circular	8.42	-0.348	1.44	4.82	-0.242	1.35	-0.106
cup.							
Mean difference							-0.024

not to be significantly different from zero ($P > 0.1$); there is thus no evidence of lack of proportionality between flow and pressure drop. The range of $\log ms_1$ in these experiments corresponds to a forty-fold change of stomatal conductance. It should be mentioned that an assumed value of α of 0.04 has been used in obtaining $\log ms_1$ as counts of upper stomata were not obtained.

(d) 'Double cup' experiments. *Estimation of m and effect of upper stomata.* These experiments had two objects; the first was to determine the resistances with and without an extra length of path through the intercellular spaces so that m , the surface resistivity of the mesophyll, could be estimated. The second object in the case of *Pelargonium* was to investigate the effects of the upper stomata upon the total resistance. Two such experiments have been carried out with *Pelargonium* and one with *Begonia*; for these the leaf chambers (Heath, 1939) have been used without the knife. The change in resistance brought about by adding in effect 6 mm. to the width of the washer ($a_2 - a_1$) is found under hypostomatous conditions by comparing the value of R_h when the

upper chambers *UI* and *UO* are closed, with that obtained when the lower outer chamber *LO* is also closed (R'_h say). In the *Pelargonium* experiments further comparisons have been made between the resistance R_a with all chambers except the porometer cup *LI* open to the outside air, and those R_h under more or less hypostomatous conditions with either the upper inner chamber *UI* alone or both the upper chambers closed. Such comparisons give information as to the effect of the upper stomata on total resistance measured. For the 'extra path' determinations the general procedure is to take readings in the following order, *LI* indicating the porometer cup and closed chambers being shown in brackets: *LI*(*UI*, *UO*); *LI*(*UI*, *UO*, *LO*); *LI*(*UI*, *UO*, *LO*); *LI*(*UI*, *UO*). The mean of the two *LI*(*UI*, *UO*, *LO*) determinations can thus be compared with the value for *LI*(*UI*, *UO*) interpolated to the same time. In order to estimate the effects of the upper stomata, other readings are taken in the order: *LI*()¹; *LI*(*UI*); *LI*(*UI*); *LI*() or: *LI*(); *LI*(*UI*, *UO*); *LI*(*UI*, *UO*); *LI*(). After opening, and especially after closing chambers, it is necessary to make sure that the new state of equilibrium has been reached before taking further readings.

Experiment of 8.5.39. Pelargonium. Clone 5. A leaf of 5½ in. diameter was cut off and attached to a water supply by the method of Gregory (1938) at 10.50 a.m., and was placed in darkness until 1.0 p.m. when it was set up in the leaf chambers. A small cup was fitted on another part of the leaf at 1.30 p.m. (see section 2 (e) below). Readings as described above were made from 4.0 p.m. while the stomata closed slightly. At 6.2 p.m. the light (described in the previous paper) was switched on and a further series of similar readings was taken when the stomata had opened considerably. A few small injected patches developed under the washers during the latter part of the experiment. The number of lower stomata within *LI* was estimated as 11,990 per cm.² Unfortunately no counts were made of upper stomata.

Experiment of 12.12.39. Pelargonium. Clone 3. An attached leaf of diameter 4½ in. on a plant grown from a June cutting was set up overnight in the leaf chambers and darkened. Readings as above were carried out from 11.8 a.m. while the stomata remained almost stationary. At 12.12 p.m. the leaf was illuminated by means of a 200 w. lamp, with water screen, about one foot distant and further readings were taken with open stomata. The strips of lower epidermis revealed that in a number of stomata the guard cells had collapsed and were apparently dead. Where such stomata appeared definitely closed and non-functional they were omitted from the count which therefore gave the low value of 7,860 per cm.² The upper stomata averaged 330 per cm.² giving a ratio of upper to lower of 0.042.

Experiment of 13.12.39. Begonia. A leaf 6½ in. long was cut off and placed in water at 10.15 a.m. It was set up in the leaf chambers with the 200 w. light on and readings were begun at 11.15 a.m. After a series of readings during which the stomata opened somewhat the leaf was darkened at 1.51 p.m. Very little stomatal closure occurred during the afternoon, so a new end was cut on the petiole and the leaf was left darkened overnight. Next morning the stomatal resistance had increased considerably and a further series of readings was taken. The number of stomata within *LI* was estimated as 6,383 per cm.²

¹ Empty brackets indicate that all chambers except *LI* were open to the outside air.

TABLE III

*Pelargonium**Double-cup Experiments. Estimation of m , the Surface Resistivity of the Mesophyll*1 unit of resistance = 3.77×10^8 cm.⁻²

Date.	$\log ms_1^*$	S_s	R_h $LI(UI, UO)$	R'_h $LI(UI, UO, LO)$	$R'_h - R_h$	m
8.5.39.	1.94	0.15	1.055	1.156	0.101	5.4
	1.94	0.15	1.060	1.156	0.096	
	0.00	0.17	0.955	1.043	0.088	
	1.33	3.64	0.173	0.308	0.135	4.88
	1.39	4.18	0.166	0.299	0.133	4.78
						Mean = 4.83
12.12.39.	0.24	0.45	0.623	0.707	0.084	4.2
	0.27	0.48	0.599	0.669	0.070	
	0.28	0.49	0.586	0.674	0.088	
	1.49	8.03	0.152	0.285	0.133	4.75
	1.52	8.60	0.149	0.286	0.137	4.87
						Mean = 4.86
13.12.39.	0.16	0.14	2.494	2.511	0.017	< 10
	0.17	0.14	2.469	2.503	0.034	
	0.17	0.14	2.443	2.494	0.051	
	0.19	0.15	2.366	2.383	0.017	—
	0.19	0.15	2.330	2.345	0.015	—
						Mean = 16.6

Begonia

Date.	$\log ms_1^\dagger$	S_s	R_h	R'_h	$R'_h - R_h$	m
13.12.39.	0.16	0.14	2.494	2.511	0.017	< 10
	0.17	0.14	2.469	2.503	0.034	
	0.17	0.14	2.443	2.494	0.051	
	0.19	0.15	2.366	2.383	0.017	—
	0.19	0.15	2.330	2.345	0.015	—
	0.93	0.80	0.871	1.206	0.335	—
	0.99	0.92	0.821	1.181	0.360	—
	1.04	1.04	0.765	1.191	0.426	—
	1.08	1.14	0.748	1.184	0.436	—
	1.13	1.27	0.715	1.163	0.448	16.5
	1.19	1.46	0.685	1.129	0.444	16.3
	1.21	1.53	0.658	1.122	0.464	16.9
						Mean = 16.6

* Derived from $\log m/R_h$. To obtain $\log s_1$ subtract 0.69.

† " " " " " " " 1.22.

The results of the above three experiments showing the effect of an extra 6 mm. width of washer are given in Table III. Within each experiment the data have been arranged in ascending order of stomatal conductance, as shown by the values of $\log ms_1$ or S_s (the conductance per 10,000 stomata) tabulated in the second and third columns. The fourth and fifth columns show the resistances R_h and R'_h found under hypostomatous conditions with the lower outer chamber open and closed respectively. The difference $R'_h - R_h$ gives

the additional resistance due to an extra 6 mm. of washer and it will be seen that this rises as the stomata open. This confirms Newton's suggestion that as the stomata open less of the air enters through those remote from the cup, for if less air is *normally* passing across the 6 mm. of extra path the increase in resistance when *LO* is closed must be greater. It also demonstrates the increasing importance of the effect of the washer with increasing stomatal conductance. The last column of the table gives values of m calculated by the method of successive approximation given in the Appendix (p. 497). This calculation has been carried out for the individual values of R'_h and R_h corresponding to the two or three largest stomatal conductances in each experiment, and means of the values of m so obtained have been taken for actual use. The remarkable agreement between the mean values of m found in the two experiments with *Pelargonium* will be noted. For the smaller stomatal openings, means of three values of R_h and the three corresponding values of R'_h have been used. Here the errors are larger for $(R'_h - R_h)$ is less accurately determined by means of the porometer. Nevertheless, for *Pelargonium* the results are in reasonable agreement with those found at larger stomatal apertures and there is no evidence of a consistent trend. The attempt to estimate m at the smallest stomatal conductances for *Begonia* failed owing to the lack of sensitivity of the porometer in terms of R at such high resistance. It will be noted that the internal resistance of the *Begonia* leaf is considerably higher than that for *Pelargonium*.

Table IV gives the results of the two experiments with *Pelargonium* showing the effect of the upper stomata. As in Table III, the second and third columns show values of $\log ms_1$ and S_g arranged in ascending order. For the experiment of 8.5.39 a stomatal ratio of 0.04 has been assumed. The resistances R_a under amphistomatous conditions are shown in the fourth column while those R_h with one or both of the upper chambers closed are given in the fifth. The difference $R_h - R_a$ and the ratio R_h/R_a given in the last two columns show respectively the absolute and relative effects upon resistance of stopping flow through the upper stomata, either above the cup only [$LI(UI)$] or over a wider area [$LI(UI, UO)$]. It will be seen that there are no consistent differences between the results obtained with one or with both of the upper chambers closed, indicating that the effect of the upper stomata outside the area immediately above the cup is relatively unimportant. This might be expected on theoretical grounds. The conditions with the upper chambers closed may therefore be considered as approximating closely to those for a hypostomatous leaf, incidentally a requisite condition for the evaluation of m . It is apparent from the results in Table IV that the effect of the upper stomata increases greatly at high stomatal conductances, as is predicted by theory. It will be noted that in the experiment of 8.5.39 the last two values of R_h show a slight increase while R_a is still falling. This may perhaps be attributed to the development of the injected patches referred to above (p. 480). These results are considered further in the Discussion below.

TABLE IV

*Pelargonium**Double-cup Experiments. Effect of Upper Stomata*1 unit of resistance = $3.77 \times 10^8 \text{ cm.}^{-3}$ Values in italics indicate that R_h was obtained with only the upper inner chamber closed [$LI(UI)$].

Date.	$\log ms_1^*$	S_s	R_a		$R_h - R_a$	R_h/R_a
			$LI($	$)$		
				$LI(UI) \text{ or } UI)$		
8.5.39.	$\bar{I} \cdot 93$	0.145	1.029	1.045	0.016	1.02
	$\bar{I} \cdot 94$	0.148	1.025	1.034	0.009	1.01
	$\bar{I} \cdot 95$	0.152	1.019	1.045	0.026	1.03
	0.02	0.178	0.872	0.883	0.011	1.01
	0.08	0.204	0.791	0.808	0.017	1.02
	1.94	14.8	0.0652	0.1591	0.094	2.44
	1.97	15.9	0.0636	0.1609	0.097	2.53
	2.03	18.2	0.0577	0.1797	0.122	3.11
12.12.39.	0.23	0.44	0.617	0.623	0.006	1.01
Ratio of	0.25	0.46	0.596	0.605	0.009	1.02
upper/lower	0.25	0.46	0.592	0.601	0.009	1.02
stomata =	0.26	0.47	0.581	0.586	0.005	1.01
0.042	0.29	0.51	0.555	0.556	0.001	1.00
	1.36	6.0	0.137	0.153	0.016	1.12
	1.39	6.4	0.132	0.152	0.020	1.15
	1.47	7.7	0.120	0.149	0.029	1.24
	1.48	7.9	0.118	0.148	0.030	1.25
	1.53	8.8	0.112	0.146	0.034	1.30
	1.54	9.0	0.111	0.144	0.033	1.30
	1.58	9.9	0.106	0.144	0.038	1.36

* These figures are derived from $\log m/R_a$, assuming $\alpha = 0.04$. To obtain $\log s_1$ subtract 0.69.

(e) *Comparison of leaf chamber and small circular cup.* Three experiments have been carried out with *Pelargonium* in which alternate readings have been taken with the leaf chamber *LI* as porometer cup on one part of a leaf and a small circular porometer cup on another part of the same leaf. In two of these the knife has been in position round the side of the outer leaf chambers nearest the edge of the leaf.

Experiment of 27.4.39. Pelargonium. Clone 5. A leaf of $5\frac{1}{4}$ in. diameter was cut off and attached to a water supply at 10.15 a.m. After being placed in the dark and left until 1.0 p.m. it was set up in the leaf chambers (with knife) and again darkened. A small cup was fixed on another part of the leaf at 4.0 p.m. and gave a very high reading at once. Readings were carried out from 4.40 p.m., the leaf being illuminated after the first twenty minutes. The estimated numbers of lower stomata were 11,570 per cm.^2 for the leaf chamber and 10,870 per cm.^2 for the small cup.

Experiment of 3.5.39. Pelargonium. Clone 5. A leaf of $5\frac{1}{4}$ in. diameter was attached to a water supply at 9.50 a.m. and set up in the leaf chambers with the knife in position at 10.15 a.m. The small cup was fitted at 11.15 a.m., but apart from this the leaf was kept in the dark until and after readings were begun at 12.11 p.m.

The leaf was illuminated after the first $38\frac{1}{2}$ minutes of readings, and the stomata opened steadily up to 100 minutes when the leaf was again darkened. Further readings were taken while the stomata closed. The numbers of lower stomata were estimated as 10,280 per cm^2 and 11,830 per cm^2 for the leaf chamber and small cup respectively.

Experiment of 8.5.39. Pelargonium. Clone 5. The setting up of this experiment has already been described above (p. 480). A number of readings was taken with the small cup, while the leaf was in darkness, for comparison with the leaf-chamber results and a series of alternate readings was taken following the switching on of the light. The lower stomata within the small cup were estimated as numbering 14,870 per cm^2 .

The results of these three experiments are presented graphically in Fig. 8. Values of S_g , the conductance per 10,000 stomata, have been calculated and their logarithms plotted against time. Unfortunately, no counts of upper stomata were made for any of these experiments, and an assumed value of α of 0.04 has been used in obtaining $\log ms_1$ from $\log m/R_2$ by means of the theoretical curves. The other constants used are indicated in the figures, except m which in all cases has been taken as 4.85.

It will be seen that there is some general agreement between the values of $\log S_g$ for the leaf chamber and small cup. During the preliminary period of darkness the stomatal behaviour is somewhat erratic. This might be due to a variety of causes such as incomplete recovery from the shock of fixing the cup, or slight leakages of light. It is probable that not much significance should be attached to the difference between the stomatal conductance for the leaf chamber and that for the small cup during this period. All three experiments agree, however, in showing at the widest stomatal apertures a somewhat higher stomatal conductance obtained from the leaf-chamber data than from the small cup. The discrepancy is no more marked with the knife (27.4.39 and 3.5.39) than without it (8.5.39) and it seems that the use of $b = 2.0$ under the former conditions is a sufficiently near approximation in the present state of accuracy of the method. The difference could be reduced but by no means annulled by assuming a much higher value of α . Thus the difference of the final values of $\log S_g$ in the 8.5.39 experiment is 0.50 (corresponding to a factor of 3 for S_g). If α is taken as 0.10, this difference is reduced to 0.34 (a factor of 2.2). Similarly, even doubling the value used for m only reduces this difference in $\log S_g$ to 0.21 (a factor of 1.6). One point in connexion with the technique should be mentioned since it will tend to cause differences in the observed sense. With the small cup, the leaf is pressed lightly against a glass or perspex plate and hence there must be some resistance to flow of air to the upper stomata. It seems improbable that this resistance will be appreciable compared with that of the upper stomata in view of the virtual impossibility of making an air-tight joint on the hairy epidermis even with a gelatine washer unless it is greased. However, such resistance as does arise from this cause will be additional to that of the upper

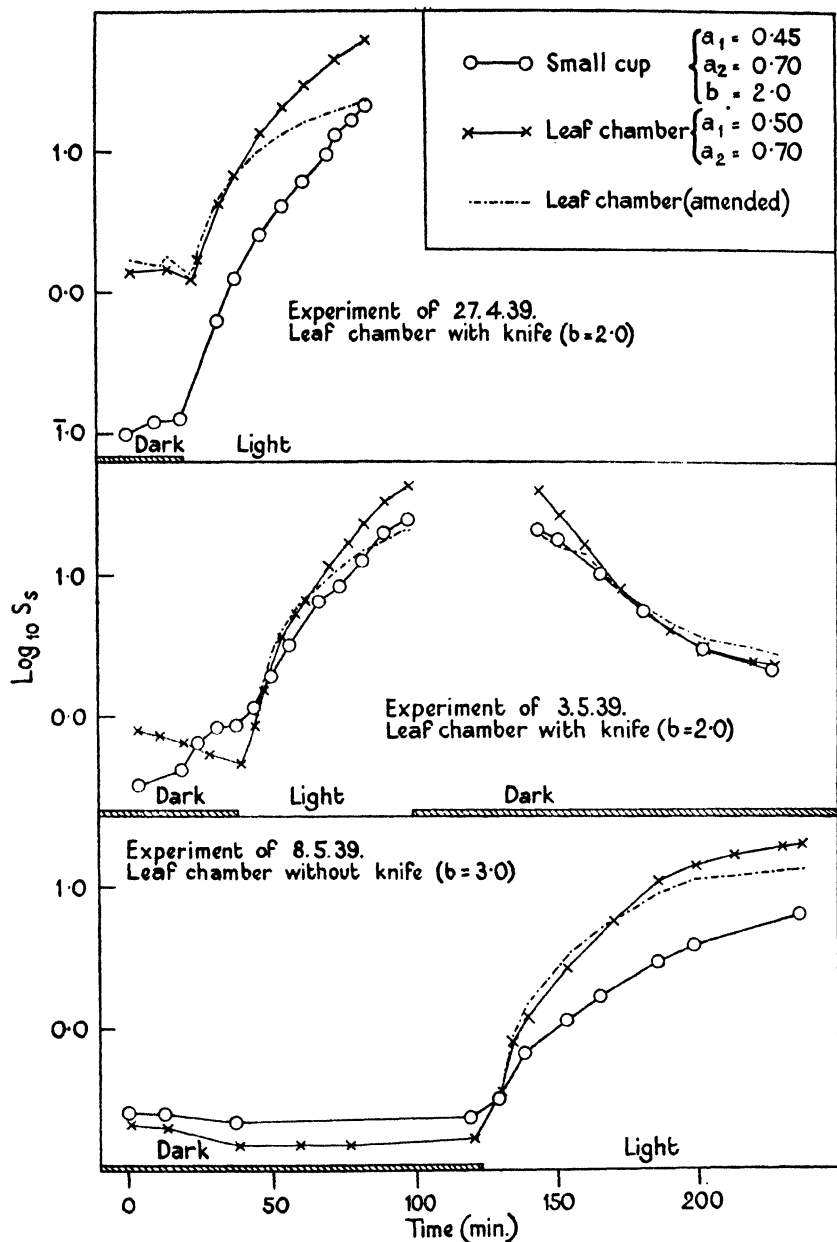


FIG. 8. *Pelargonium*. Comparison of the values obtained with the leaf chamber *LI* and a small circular cup during stomatal movement. Logarithms of conductance S_s per 10,000 stomata of the lower surface.

stomata and hence will have an effect somewhat similar to a reduction in their number. No such resistance occurs in the case of flow to the upper stomata above *LI*. Considering again the final values in the experiment of date 8.5.39 it would be necessary to assume $\alpha = 0.10$ for the leaf chamber and $\alpha = 0.00$ for the small cup in order to remove the whole of the discrepancy. It would appear that the difference between S_g for *LI* and for the small cup must be attributed at least in part either to actual differences in stomatal aperture or to inadequacy in the theory for the elongated cup, since this is certainly much less precise than that for a circular cup. The consistent differences found at wide apertures in all three experiments suggest that the latter is more likely to be the cause. This question is considered further in the Discussion below, where an explanation of the 'amended values' in Fig. 8 will be found.

3. Discussion

The conclusions to be drawn from the available experimental evidence bearing upon the adequacy of the theory for an elongated cup may now be considered. The experiments in which the leaf chambers are used as a double cup to estimate m for *Pelargonium* under hypostomatous conditions agree in giving an approximately constant value for m over a considerable range of stomatal conductance. This range is equivalent to a change in $\log ms_1$ from 0.0 to at least 1.5 (Table III), and if $\log ms_1$ is calculated from m/R_a with an assumed value for α of 0.04 (as in Table IV) the uppermost value of $\log ms_1$ is estimated as 2.0. This constancy of m would appear to provide evidence that the *shape* of the $\alpha = 0$ curve relating $\log m/R_2$ to $\log ms_1$ is approximately correct, although as mentioned earlier (p. 476) the existence of an error acting as a multiplying constant may displace the curve.

The second test suggested in section 1 (*f*) is illustrated in Fig. 9. At chosen values of $\log ms_1$ the differences $\log m/R_a - \log m/R_h$ (i.e. $\log R_h/R_a$) have been read off from the theoretical curves relating $\log m/R_2$ to $\log ms_1$ for several values of α . These differences are plotted against $\log m/R_a$ to give a series of theoretical difference-curves. From the data for the two experiments with *Pelargonium* in which the effect of the upper stomata was investigated, observed values of $\log R_h/R_a$ have also been plotted against $\log m/R_a$ using the value of m actually found for these leaves, namely 4.85. Since the observed points from both experiments seem to lie upon a smooth curve, a single free-hand curve has been drawn through them. The observed stomatal ratio for the experiment of 12.12.39 is 0.04, that for the experiment of 8.5.39 being unknown but likely to be similar. It will be seen that there is a similarity both of form and position between the observed and calculated curves which provides some general confirmation of the theory, especially as the test is obviously an extremely sensitive one. But since the divergence from theory is evidently real it is necessary to inquire which of the many assumptions involved in the theory may be at fault. Neglect of the end effects in the leaf

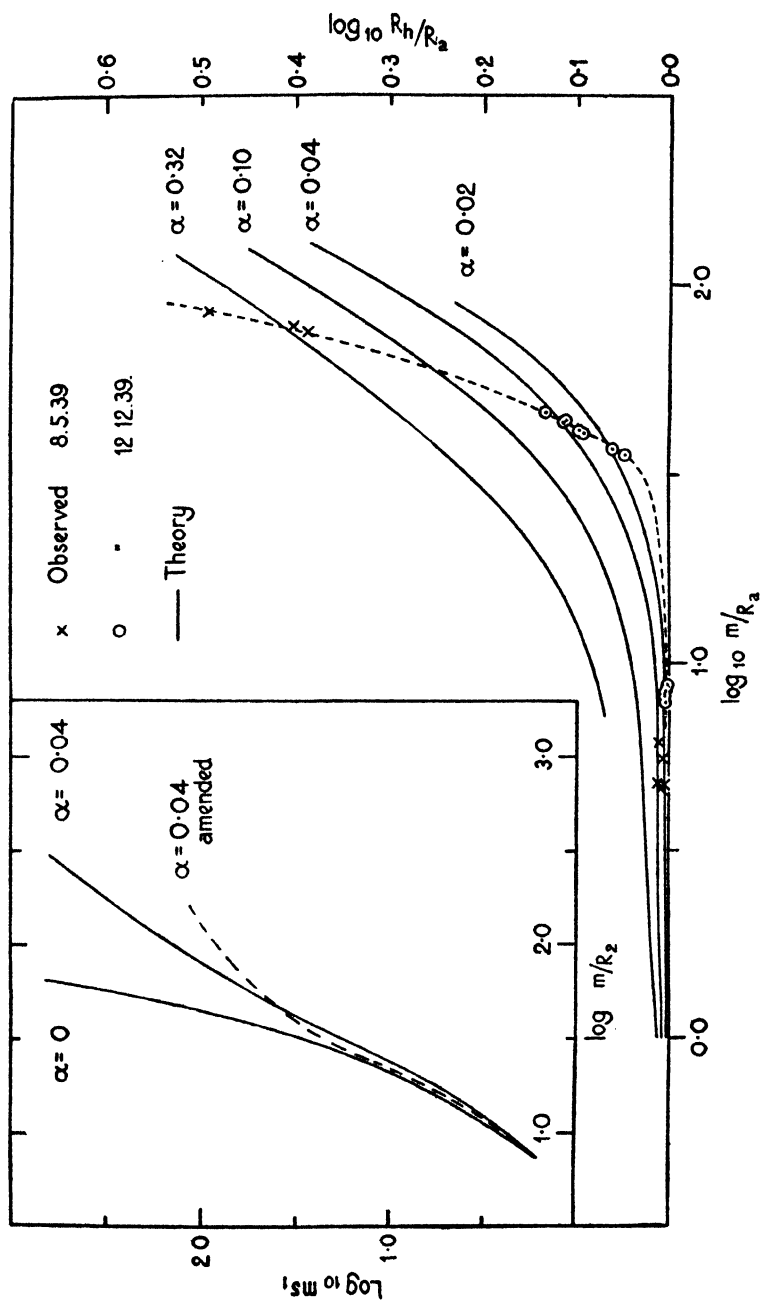


FIG. 9. For explanation see text.

chamber will mean that at wide stomatal apertures, when the washer has much more effect upon $\log m/R_h$ than upon $\log m/R_a$, the theoretical values of $\log R_h/R_a$ are too large. Correcting this error, which is unlikely to be a large one since the width of *LI* is only 11 per cent. of the length, would make the fit even worse. Even large errors in *b* have practically no effect at medium and large stomatal apertures. Errors in *m*, if constant, would affect the position but not the shape of the observed curve. It has been shown experimentally that *m* is approximately constant over the range of stomatal conductance under discussion. If the resistance to flow through the palisade tissue was appreciable, this would be additional to the resistance of the upper stomata and hence of most importance at large stomatal conductances. It would then have an effect similar to a decrease in α . Allowance for this would make the corrected theoretical difference-curves lower and the fit worse. There is, however, one assumption which if incorrect might cause a deviation from theory in the direction observed, namely that the ratio α of the conductances s_1 and s_2 is constant and that it is the same as the ratio of the stomatal numbers. Should the upper stomata open in the light much more rapidly than the lower, owing to the higher light intensity or difference in quality, α would increase as the stomata opened. This would give a difference in the curves similar to that found, e.g. suppose that in the experiment of 8.5.39 the stomatal ratio were 0.04 and that α had this value in the dark at $\log m/R_a = 0.7$. If the upper stomata were to open more rapidly in the light, so that at a certain stage they were twice as widely open as the lower, their relative conductance might be expected to increase about 2^3 times and α would now be 0.32. A test of this hypothesis could be carried out by repeating the experiments with illumination from below instead of from above. If the lower stomata then opened more rapidly than the upper, the observed curve should have a trend away to the right of the theoretical one. It is hoped to make this test at a future date, but it must be pointed out that, with either type of illumination, as both upper and lower stomata approach their full aperture the observed curve should first turn back towards the theoretical curve and ultimately run parallel with it. No tendency for this to occur is shown in the present experiments, despite the fact that in both cases the stomata were almost stationary after a long period of illumination when the observations at the widest apertures were made. In the case of the experiment of 8.5.39 the lack of tendency for the curve to bend over might be attributed to the small injected patches under the washer (see pp. 480 and 482) which would tend to increase R_h/R_a . No such explanation is applicable to the experiment of 12.12.39. The assumption referred to above as a possible cause of the discrepancy between observation and theory implies that interference between the stream-lines flowing through neighbouring stomata is negligible. Such interference will not only increase in amount as the stomata open and are therefore separated by fewer diameters, but it will certainly be much more important for the lower than for the widely separated upper

stomata. Its effect if appreciable will be to cause an increase of α as the stomata open, thus tending to give the observed result. This explanation, however, seems unlikely to apply to the discrepancy between the results for the leaf chamber and a small cup discussed in the next paragraph. It will be appreciated that it is by no means obvious where the inadequacy lies in the theory for an elongated cup. As indicated in the previous paragraph, the theory for a hypostomatous leaf would appear to be either adequate or else invalid owing to a multiplying constant only (e.g. an error in the effective length of the chamber l). It is apparent, however, that the theory for an amphistomatous leaf would be improved by being amended in some respects. Some indication of the type of amendment needed is shown in the inset to Fig. 9. Here parts of the theoretical $\alpha = 0$ and $\alpha = 0.04$ curves relating $\log m/R_2$ to $\log ms_1$ are shown for the leaf chamber *LI* ($a_1 = 0.5$; $a_2 = 0.7$; $l = 9.0$; $b = 3.0$). Assuming the $\alpha = 0$ curve to be the more reliable, values of $\log R_h/R_a$ have been read off from the observed curve at chosen values of $\log m/R_a$ and added to the values on the calculated $\alpha = 0$ curve at the same $\log ms_1$. Hence an amended $\alpha = 0.04$ curve (shown as a broken line) has been obtained for $\log m/R_2$ v. $\log ms_1$. The part of this curve where $\log m/R_2$ is greater than 1.7 should be regarded with caution, depending as it does upon those results of the 8.5.39 experiment in which injection may have affected the observed value of $\log R_h/R_a$.

The experiments comparing stomatal conductances obtained with the leaf chamber and small cup respectively also indicate that the theory for an elongated cup needs some amendment, if it is assumed that the theory for a circular cup is correct. The type of correction apparently needed is such as would be given by an increasing value of α as the stomata open, i.e. the type of amended curve shown in the inset to Fig. 9. It should be noted that here the discrepancy is unlikely to be caused by interference between flow through neighbouring stomata, or by the upper stomata opening more rapidly in the light, for these factors should affect similarly both the small cup and leaf chamber results. Making use of the amended curve (Fig. 9), the values of $\log S_g$ for the leaf chamber have been recalculated for each of the three experiments and curves of amended values plotted in Fig. 8. It will be noted that in all cases there is a marked improvement in the agreement at large apertures with the results for the small cup, indicating that this is approximately the type of correction needed. For the present, however, the theory for the elongated cup will continue to be used in its original form, which as the experimental tests show is a useful approximation.

It is realized that the experimental data are very meagre considering the extent of theory involved, and much further work will be needed. The paucity of data is the result of the experimental work being carried out before the theoretical aspects of the problem were elucidated. The fortunate collaboration of Dr. Penman eventually made this possible, but at a time when further experimentation was interrupted by the war.

SUMMARY

A theoretical study of the resistance porometer method is presented, together with the results of an experimental investigation of the adequacy of the theory formulated. The theoretical considerations are first dealt with and the experimental evidence is later presented and discussed. The mathematical treatment of the theory is presented in an appendix by Dr. H. L. Penman.

Previous attempts to calibrate porometers are briefly reviewed.

The structure of the leaves used (*Pelargonium zonale* and *Begonia sanguineum*) is described with reference to the paths of flow of gas during porometer experiments.

The methods of calculation of flow resistance or conductance from porometer readings are described, the conditions for their valid use are considered, and the theoretical limitations involved are discussed in detail.

The sensitivity of the resistance porometer is considered, and it is concluded that from a statistical point of view the use of logarithmic values of resistance and conductance is to be preferred.

The total resistance or conductance as estimated with the porometer is analysed into that due to (1) the stomata within the cup and (2) the mesophyll and the remaining stomata. Previous work along these lines is discussed. The consequences of the theoretical treatment of this relation are considered (a) for a circular porometer cup and (b) for elongated cups. For the latter the theory is not precise and therefore experimental tests of its validity are given.

The principal condition necessary for the valid estimation of leaf resistance is proportionality between flow and pressure difference. Experimental confirmation of this condition is presented.

By the use of the leaf chambers (Heath, 1939) as a 'double cup' the value of the surface resistivity (m) of the mesophyll is estimated. These experiments also confirm certain predictions based on the theoretical investigation, namely the increasing importance with stomatal opening of the width of the washer attaching the porometer cup to a hypostomatous leaf, and also to some extent the form of the theoretical curve relating total resistance and stomatal conductance for such a leaf.

During these experiments with the leaf chambers the effects of the upper stomata upon total resistance have been investigated by opening and closing the upper chambers to the outer air. In this way the increasing importance of the upper stomatal effect with increasing aperture is demonstrated, thus confirming another prediction from the theoretical investigation. It is shown that with the upper chambers closed the conditions approximate closely to those for a hypostomatous leaf, this being a requisite condition for the estimation of m .

Experiments with the leaf chambers and a small circular porometer cup on the same leaf show that there is general agreement in the values of stomatal conductance obtained from the respective theories for these two types of cup.

Nevertheless, the results at wide stomatal apertures indicate that the theory for an elongated cup requires some amendment.

The relevant experimental evidence for the need of such amendment and the type of correction required are discussed.

The author has great pleasure in thanking Dr. H. L. Penman, both for working out the new theories presented in the Appendix and for many other helpful suggestions and criticisms. He is also most grateful to Professor F. G. Gregory for his continued and stimulating interest in this work. Other acknowledgements are made in the text. Part of the work was carried out while the author was holding a Leverhulme Research Fellowship.

APPENDIX

Theory of Viscous Flow Porometers

BY

H. L. PENMAN

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Each of the experimental systems requires separate treatment. The theory of the circular cup, used also for diffusion, is being published elsewhere, and the first part of this appendix is a very brief sketch of the theory of viscous flow; it consists essentially of the equations needed for calculation, and little else. For the leaf chambers more detail is supplied, and the analysis given for these will indicate the principles and methods used in obtaining the results for the circular cup.

I. Theory of viscous flow with circular porometer cup.

The leaf, or part of the leaf, is assumed to be circular and bounded by an impervious wall around the circumference. Stomatal perforations in each of the surfaces permit the flow of fluids through the epidermis and it is assumed that mean sizes of the two sets are equal, and that mutual interference between the stream-lines of flow through neighbouring stomata is either non-existent or affects both sets equally; hence the ratio of the numbers per unit area gives the conductivity ratio. The porometer cup is supposed applied symmetrically about the centre of the lower epidermis. The leaf is supposed so thin that there is no resistance to flow across the thickness, but there is resistance to flow within the leaf parallel to the plane of the leaf. It is assumed that pressure gradients are everywhere small so that changes in density can be neglected. In any self-consistent set of units, let

- b = radius of disc of leaf (cm.),
- a_2 = radius of outer edge of cup,
- a_1 = radius of inner edge of cup,

- s_1 = conductivity of lower epidermis (per sq. cm.),
 s_2 = conductivity of upper epidermis (per sq. cm.),
 m = superficial resistivity of mesophyll (per cm./cm.),
 p_0 = pressure difference between outside air and inside cup,
 i = flow produced (per sec.),
 R = measured resistance,
 p = difference between pressure at radius r inside the leaf and that inside the cup.

Flow equation. By considering an annular ring, radius r width δr , flow

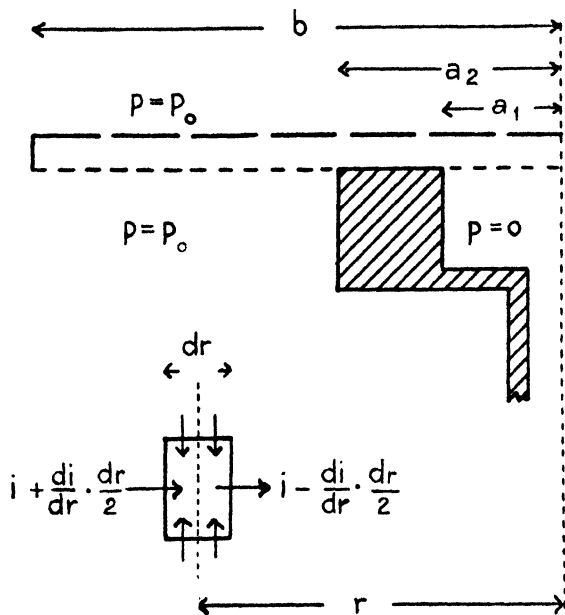


FIG. 10. Idealized half section of leaf and porometer cup.

equations for the parts of the leaf (i) outside the cup, (ii) inside the cup, and (iii) under the washer, can be set up. These are all of the same form and the solutions are:

$$b > r > a_2, \quad p_0 - p = AI_0(\rho_1) + BK_0(\rho_1), \quad (1)$$

$$a_1 > r > 0, \quad \frac{s_2 p_0}{s_1 + s_2} - p = CI_0(\rho_1) + DK_0(\rho_1), \quad (2)$$

$$a_2 > r > a_1, \quad p_0 - p = FI_0(\rho_2) + GK_0(\rho_2), \quad (3)$$

where

$$\rho_1 = r\sqrt{\{m(s_1 + s_2)\}} = r\beta_1, \quad \text{say,}$$

$$\rho_2 = r\sqrt{(ms_2)} = r\beta_2,$$

$I_0(\rho)$ and $K_0(\rho)$ are Bessel functions,¹ and A, B, C, D, F , and G are constants

¹ The tables employed in this part of the work are B.A. Mathematical Tables; Bessel Functions, Part I (Cambridge Univ. Press, 1937).

to be determined from boundary conditions. These boundary conditions are: (i) at $r = 0$, $p \neq \infty$; (ii) at $r = b$ there is no flow across the boundary; (iii)–(vi) at $r = a_2$, and $r = a_1$, the values of p and dp/dr are continuous.

Applying these conditions we obtain:

$$\frac{\beta_2}{\beta_1} \left[\frac{K_1(\beta_1 b) I_0(\beta_1 a_2) + I_1(\beta_1 b) K_0(\beta_1 a_2)}{K_1(\beta_1 b) I_1(\beta_1 a_2) - I_1(\beta_1 b) K_1(\beta_1 a_2)} \right] = \frac{(F/G) I_0(\beta_2 a_2) + K_0(\beta_2 a_2)}{(F/G) I_1(\beta_2 a_2) - K_1(\beta_2 a_2)}, \quad (4)$$

$$\frac{\beta_2}{\beta_1} \left[\frac{C I_0(\beta_1 a_1) + \frac{s_1 p_0}{s_1 + s_2}}{C I_1(\beta_1 a_1)} \right] = \frac{(F/G) I_0(\beta_2 a_1) + K_0(\beta_2 a_1)}{(F/G) I_1(\beta_2 a_1) - K_1(\beta_2 a_1)}. \quad (5)$$

Thus for any assumed value of β_1 , given the dimensional constants b , a_1 , and a_2 , and the ratio s_2/s_1 ($= \alpha$, say), the value of C can be found from the second of these equations, the first being used to evaluate F/G .

The total flow through the cup, i , is given by p_0/R , where R is the measured resistance. We find

$$i = \frac{p_0}{R} = \frac{2\pi s_1}{m(s_1 + s_2)} \left[\frac{s_2 p_0}{s_1 + s_2} \frac{\beta_1^2 a_1^2}{2} - C \beta_1 a_1 I_1(\beta_1 a_1) \right]. \quad (6)$$

Actually, for computational purposes, we can substitute from (5) in (6). Putting the right-hand side of (5) equal to N , we obtain

$$\frac{m}{R} = \frac{2\pi\beta_1 a_1}{(1 + \alpha)^2} \left[\frac{\alpha}{2} \beta_1 a_1 + \frac{1}{I_0(\beta_1 a_1) - N \sqrt{\frac{1 + \alpha}{\alpha}}} \frac{1}{I_1(\beta_1 a_1)} \right]. \quad (6a)$$

Also $\beta_1^2 = m(s_1 + s_2)$, i.e. $m s_1 = \beta_1^2/(1 + \alpha)$.

Thus for any assumed value of β_1 and α , values of m/R and $m s_1$ can be calculated.

Special case. When $\alpha = 0$ (hypostomatous conditions), several terms in (4) and (5) become either zero or infinity. In this case, the solution is a little simpler, and putting the large square bracket of the left-hand side of (4) equal to M , we obtain

$$\frac{2\pi R}{m} = \log_e \frac{a_2}{a_1} - \frac{M \frac{a_1}{a_2} - I_0(\beta_1 a_1)}{\beta_1 a_1 I_1(\beta_1 a_1)}. \quad (7)$$

The curves of Fig. 11 have been derived in this way, using equations (4), (5), and (6) for $\alpha = 0.04$ and $\alpha = 0.10$, and using equation (7) for $\alpha = 0.00$. The values of β_1 used are such that $\beta_1 a_1 = 0.05, 0.20, 0.80, 1.60, 3.20, 6.40, 12.80$, and 20.0 . Other constants used in Fig. 11 are $b = 2.0$ cm.; $a_2 = 0.85$ cm., $a_1 = 0.45$ cm. To obtain a value of s_1 for an observed R it is necessary to know m .

Limiting values. (i) *Small stomatal aperture.* For all small values of α ,

including $\alpha = 0$, we find that if $b^2 - a_2^2$ is large compared with a_1^2 , then, as $\beta_1 \rightarrow 0$,

$$1/R \rightarrow \pi a_1^2 s_1, \quad (8)$$

or all the resistance is due to the lower epidermis under the cup.

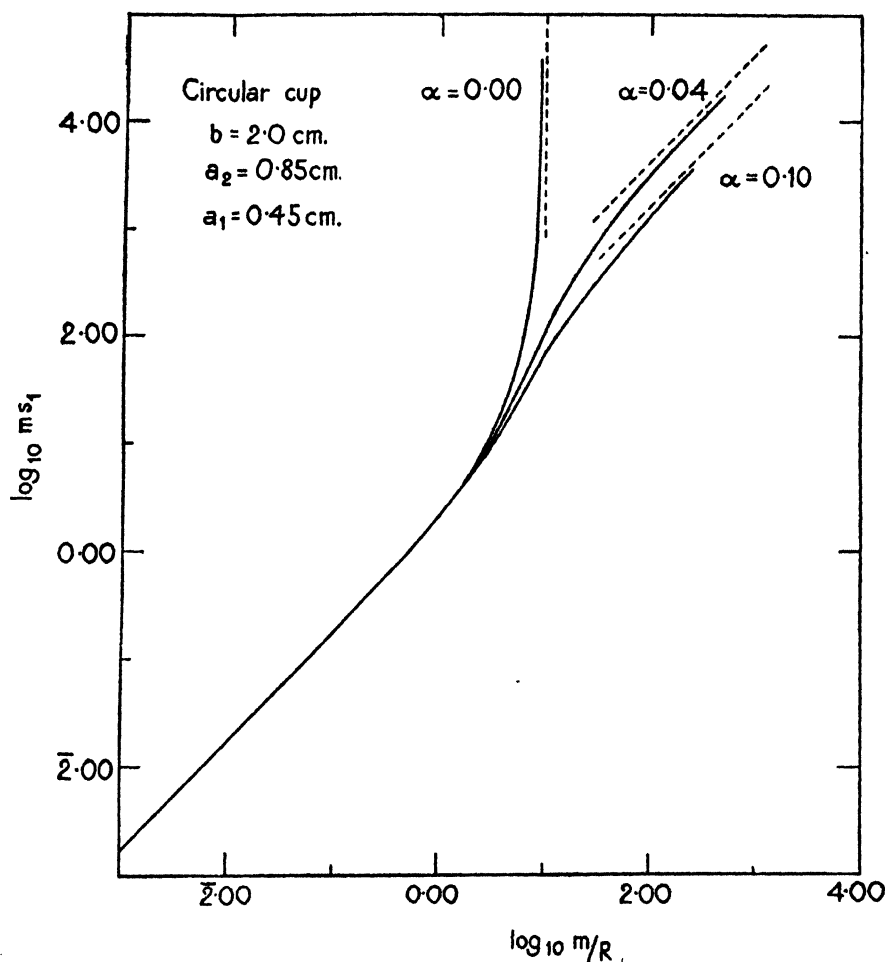


FIG. 11. The relation between total resistance R and stomatal conductance s_1 .

(ii) *Large stomatal aperture.* When $\alpha \neq 0$, as $\beta_1 \rightarrow \infty$, we find

$$R \rightarrow \frac{1}{\pi a_1^2 s_1} + \frac{1}{\pi a_2^2 s_2}, \quad (9)$$

i.e. the resistance is wholly due to the resistances of the upper and lower parts of the leaf under the cup. When $\alpha = 0$, we obtain from equation (7), as $\beta_1 \rightarrow \infty$

$$R \rightarrow \frac{m}{2\pi} \log_e \frac{a_2}{a_1}. \quad (10)$$

The asymptotes given by (9) and (10) have been included in Fig. 11.

Determination of mesophyll resistance. To determine m let us suppose that the upper epidermis is closed ($s_2 = 0$) and everything kept constant except a_2 which is increased to a_3 . With an obvious symbolism, M_2 becomes M_3 , R_2 becomes R_3 , and putting $I_0(\beta_1 a_1)/\beta_1 a_1 I_1(\beta_1 a_1) = \gamma$, we have from (7),

$$R_2 = -\frac{m}{2\pi} \left[\frac{M_2}{a_2 \beta_1} - \gamma - \log_e \frac{a_2}{a_1} \right],$$

$$R_3 = -\frac{m}{2\pi} \left[\frac{M_3}{a_3 \beta_1} - \gamma - \log_e \frac{a_3}{a_1} \right],$$

$$R_3 - R_2 = -\frac{m}{2\pi} \left[\frac{1}{\beta_1} \left(\frac{M_3}{a_3} - \frac{M_2}{a_2} \right) - \log_e \frac{a_3}{a_2} \right]. \quad (11)$$

Assume, as a first approximation, that the first term in the bracket is negligible: we have an approximate value of m , m_1 say, $= \frac{2\pi(R_3 - R_2)}{\log_e a_3/a_2}$. Using this value of m_1 and the known value of R_2 , $m_1 s_1 (= \beta_1^2)$ can be read from the curve for $\alpha = 0$, i.e. values of $\beta_1 a_1$, $\beta_1 a_2$, and $\beta_1 a_3$ can be found. Thus values of M_3 and M_2 can be estimated, leading to a more exact estimate of m . Further discussion of the importance of m and an alternative method of measuring it will be found below (p. 498).

II. Theory of viscous flow with leaf chambers (Heath, 1939, p. 476).

The shape of the system involved here does not possess any centre of symmetry similar to the circular cup porometer and an equally rigorous analysis is not possible. It is proposed to treat it as a rectangle of length equal to the area/width ignoring end effects. This can be partly justified for the following reasons: (1) For the inner cup at least, the length is great compared with the width; (2) veins in the leaf generally cross the walls normally, i.e. the predominant flow into the cup is normal to the walls and we can regard the system as a series of approximately rectangular systems separated by veins preventing flow parallel to the main walls.

The symbolism of the preceding section will be used again, with the modification that a_1 becomes the half width of the cup, and the section of Fig. 10 is now to be regarded as that of a strip of length l normal to the plane of the paper. The elementary area is $l \delta x$, and the general expression for the current at any distance x is $i = (l/m)(dp/dx)$. With these modifications the formal equation becomes

$$\frac{d^2 \phi}{dx^2} = \beta^2 \phi,$$

and in the appropriate ranges we have as solutions,

$$\begin{aligned} b > x > a_2, \quad p &= p_0 - (Ae^{\beta_1 x} + Be^{-\beta_1 x}), \\ a_2 > x > a_1, \quad p &= p_0 - (Fe^{\beta_1 x} + Ge^{-\beta_1 x}), \\ a_1 > x > 0, \quad p &= \frac{p_0 s_2}{s_1 + s_2} - (Ce^{\beta_1 x} + De^{-\beta_1 x}). \end{aligned}$$

At $x = 0$ we assume that $dp/dx = 0$, leading to $C = D$. The other boundary conditions are as before, namely $dp/dx = 0$ at $x = b$, and continuity of p and dp/dx at $x = a_2$ and $x = a_1$. We obtain

$$\begin{aligned} Ae^{\beta_1 b} - Be^{-\beta_1 b} &= 0, \\ Ae^{\beta_1 a_2} + Be^{-\beta_1 a_2} &= Fe^{\beta_1 a_2} + Ge^{-\beta_1 a_2}, \\ \beta_1(Ae^{\beta_1 a_2} - Be^{-\beta_1 a_2}) &= \beta_2(Fe^{\beta_1 a_2} - Ge^{-\beta_1 a_2}), \\ C(e^{\beta_1 a_1} + e^{-\beta_1 a_1}) + \frac{p_0 s_1}{s_1 + s_2} &= Fe^{\beta_1 a_1} + Ge^{-\beta_1 a_1}, \\ \beta_1 C(e^{\beta_1 a_1} - e^{-\beta_1 a_1}) &= \beta_2(Fe^{\beta_1 a_1} - Ge^{-\beta_1 a_1}), \end{aligned}$$

$$\text{and} \quad i = p_0/R = \int_{-a_1}^{a_1} l s_1 p \, dx = l s_1 \left[\frac{2a_1 p_0 s_2}{s_1 + s_2} - \frac{2C}{\beta_1} (e^{\beta_1 a_1} - e^{-\beta_1 a_1}) \right].$$

$$\text{Putting} \quad e^\theta + e^{-\theta} = 2 \cosh \theta, \quad \text{and} \quad e^\theta - e^{-\theta} = 2 \sinh \theta,$$

the general solution of these equations can be taken a little further than the corresponding earlier set and we obtain

$$\begin{aligned} \frac{\beta_2}{\beta_1} \frac{2C \cosh \beta_1 a_1 + \frac{p_0 s_1}{s_1 + s_2}}{2C \sinh \beta_1 a_1} &= -N, \quad \text{say,} \\ &= -\frac{\beta_2 \cosh \beta_1 (b - a_2) \cosh \beta_2 (a_2 - a_1) + \beta_1 \sinh \beta_1 (b - a_2) \sinh \beta_2 (a_2 - a_1)}{\beta_2 \cosh \beta_1 (b - a_2) \sinh \beta_2 (a_2 - a_1) + \beta_1 \sinh \beta_1 (b - a_2) \cosh \beta_2 (a_2 - a_1)}, \end{aligned}$$

evaluation of which for an assumed value of β_1 , and known values of α , b , a_2 , and a_1 , merely involves a straightforward use of standard tables of \sinh and \cosh . Knowing N , we have

$$p_0/R = l s_1 \left[\frac{2a_1 p_0 s_2}{s_1 + s_2} - \frac{2C}{\beta_1} 2 \sinh \beta_1 a_1 \right]$$

$$\text{or} \quad 1/R = \frac{2l s_1}{(1 + \alpha)} \left[\alpha a_1 + \frac{\beta_2}{\beta_1 \beta_2 \coth \beta_1 a_1 + \beta_1 N} \right].$$

This expression can be reduced to

$$1/R = \frac{2l}{m(1 + \alpha)^2} \left[\alpha \beta_1^2 a_1 + \frac{\beta_2}{\beta_1 \coth \beta_1 a_1 + N} \right], \quad (12)$$

giving an expression for m/R for each assumed value of β_1 . Also

$$\beta_1^2 = m(s_1 + s_2) = ms_1(1 + \alpha),$$

i.e. $ms_1 = \beta_1^2 / (1 + \alpha).$

Special case: α (or β_2) = 0. As $\beta_2 \rightarrow 0$ the value of N approaches

$$\frac{\beta_2}{\beta_1} \coth \beta_1(b - a_2) + \beta_2(a_2 - a_1),$$

and we have, for $\beta_2 \div 0$,

$$1/R = \frac{2l}{m(1 + \alpha)^2} \left[\alpha \beta_1^2 a_1 + \frac{\beta_1 \beta_2}{\beta_2 \{ \coth \beta_1 a_1 + \coth \beta_1(b - a_2) + \beta_1(a_2 - a_1) \}} \right],$$

i.e. for $\alpha = 0$ we obtain

$$1/R = \frac{2l}{m} \left[\frac{\beta_1}{\coth \beta_1 a_1 + \coth \beta_1(b - a_2) + \beta_1(a_2 - a_1)} \right], \quad \text{where } \beta_1^2 = ms_1. \quad (13)$$

Limiting values. (i) *Small stomatal aperture.* We find for all values of α that as $\beta_1 \rightarrow 0$

$$m/R \rightarrow \frac{2ls_1 m}{(1 + \alpha)} \left[\alpha a_1 + \frac{1}{\frac{1}{a_1} + \frac{1}{b - a_2}} \right],$$

i.e. if b is very large compared with a_2 and a_1 we have $1/R \rightarrow 2ls_1 a_1$ for all α , i.e. the total resistance is entirely due to the part under the cup.

(ii) *Large stomatal aperture.* When β_1 becomes very large ($\beta_2 \neq 0$) the limiting value is similarly found to be

$$m/R \rightarrow \frac{2ls_1}{s_1 + s_2} a_1 ms_1, \quad \text{or} \quad R \rightarrow \frac{1}{2la_1 s_1} + \frac{1}{2la_1 s_2},$$

i.e. the resistance is wholly due to the resistances of upper and lower epidermis under the cup. In the case of α (and β_2) = 0 the limiting value is given from (13) by

$$1/R = \frac{2l}{m} \left[\frac{\beta_1}{2 + \beta_1(a_2 - a_1)} \right],$$

i.e.
$$= \frac{2l}{m(a_2 - a_1)}; \quad m/R = \frac{2l}{a_2 - a_1}. \quad (14)$$

The resistance here is entirely due to the two parts of the mesophyll under the washer, the resistances being in parallel.

Plotting $\log ms_1$ against $\log m/R$ for various values of α gives a series of curves similar to that of Fig. 11.

Determination of mesophyll resistance. An increase in the width of the washer, in practice the addition of the outer leaf chamber, will, under hypostomatous

conditions, lead to an expression similar to equation (13) which, inverted, we may write:

$$R' = \frac{m}{2l'\beta_1} \left[\coth \beta_1 a_1 + \coth \beta_1 (b - a_3) + \beta_1 (a_3 - a_1) \right] \quad (13')$$

$$R = \frac{m}{2l\beta_1} \left[\coth \beta_1 a_1 + \coth \beta_1 (b - a_2) + \beta_1 (a_2 - a_1) \right]. \quad (13)$$

As before, if the stomata are wide open, i.e. β_1 is large, a first approximation to m is given by

$$\frac{R' - R}{m} = \frac{a_3 - a_1}{2l'} - \frac{a_2 - a_1}{2l}, \quad (15)$$

so that an approximate value of β_1 can be determined to be used in (13 and 13') to give a better value of m . If (15) is used without further correction the choice of a value for l' is important. End effects on the broad arc of the outer leaf chamber cannot be neglected, and some measure of compensation will be achieved by taking l' as the mean of l and the value obtained from the area of the outer chamber divided by its width ($2a_3$). The constants $a_3 - a_1 = 0.8$, $a_2 - a_1 = 0.2$, $l = 9.0$, $l' = \frac{1}{2}(9.0 + 10.6)$, lead to $(R' - R)/m = 0.030$, which is sufficient to give a rough value of m .

III. *Some general considerations.*

(i) *Units.* Physically, the 'resistance' of a system is the ratio of the driving potential difference to the current it produces; the preceding analysis has been based on this conception. Actually, however, the interrelations of R , m , and s_1 are such that any unit of resistance can be used. The usual resistance to fluid flow arises partly from the nature of the fluid itself and partly from the geometry of the system through which it flows, and the analysis will apply equally well to this latter part of the resistance. In this case a value of s_1 will represent $\sum_n \frac{r^4}{l}$, where n is the number of stomata per unit area and r and l are the radius and length of the equivalent capillary tube which would have the same resistance.

(ii) *Resistance across the leaf and m .* Neglect of the former seems reasonable in view of the extreme thinness of the leaf. If m is precisely known a check on this assumption is possible by measuring first the value of R_a under amphistomatous conditions and then sealing the upper surface to obtain R_h under hypostomatous conditions. If the resistance across the leaf is negligible the two values of ms_1 ought to agree. As we shall see below, the range of m/R in which this experiment could be usefully employed is one in which the accuracy of m is important and it might be more effective to use the experiment to determine m , assuming the resistance across the leaf is negligible. From the theoretical curves a derived curve of R_h/R_a against m/R_a can be plotted; from

the experiment a value of R_h/R_a is found, hence a value of m/R_a , which with the known value of R_a leads to a value of m .

The importance of m can be shown as follows. We have, at any point on the curves of Fig. 11,

$$\frac{\Delta \log ms_1}{\Delta \log m/R} = \mu \quad \text{say,}$$

from which, ignoring the constant factor which appears in both sides of the equation, we obtain

$$\frac{1}{ms_1} [s_1 \Delta m + m \Delta s_1] = \mu \frac{\Delta m}{m},$$

or

$$\Delta s_1/s_1 = (\mu - 1)\Delta m/m.$$

Thus when the slope is unity or near it the value of m is not very critical, but as μ approaches 2 the accuracy of m must be equal to the desired accuracy in s_1 . For $\mu > 2$ the accuracy of m becomes even more important and with the approximate treatment necessarily involved in discussing the leaf chambers it is doubtful whether results from such parts of the curve ought to be used without some independent confirmation of the value of m .

(iii) *Limitations of the analysis.* The discussion of the leaf chambers is not as precise as that of the circular cup. For the narrow inner chamber the error is probably of the order of a few per cent.; this is an opinion and not a reasoned judgement. For the outer chamber, where the ratio of length to width is much smaller, one can only hope that the various sources of error do not all act in the same sense and that the final error is not serious. As this outer chamber has been used in the determination of m , every possible check on the result should be employed. The basic trouble is that experimental requirements and ease of analysis are mutually conflicting and a compromise must be found between obtaining results which cannot be interpreted and obtaining no results which can be interpreted with precision!

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FERTILIZER POLICY IN WAR-TIME: THE FERTILIZER REQUIREMENTS OF ARABLE CROPS

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Summary.—In order to formulate a flexible fertilizer policy which will ensure maximum agricultural production and make the best use of available fertilizer supplies, all published results of one-year fertilizer experiments conducted since 1900 in Great Britain on the main arable crops, and also of similar series of experiments in other northern European countries, have been summarized.

The problem of determining the best dressings for the different crops under varying conditions is discussed, and rules are given for the allocation of a given amount of fertilizers to the different crops which are applicable both for a single farm and for the whole country.

The main conclusions are as follows:

1. The responses to phosphate and potash are substantially reduced when dung is applied, but crops are equally responsive to inorganic nitrogen on dunged and undunged land. Consequently smaller dressings of phosphate and potash are required on dunged land, but no reduction should be made in the nitrogenous dressing.

2. The current level of nitrogenous manuring, both in absolute amount and relative to that of the other fertilizers, is too low, particularly where dung is also given. Considerable increases in agricultural production would result from the greater use of nitrogen, especially on cereals.

3. Additional phosphate is needed for root crops, especially in the wetter districts and on phosphate-deficient soils, including much of the newly ploughed grass-land.

4. The general policy of making fertilizers in short supply available only for the most responsive crops (already adopted in the case of potash) is the correct one. It appears, however, that potatoes should receive some potash, even in the presence of dung, unless supplies are very short.

5. The responses to phosphate and potash (in contrast to nitrogen) vary markedly with soils and districts. Consequently, in order to ensure the most efficient utilization of soil reserves and fertilizer supplies, local knowledge or soil analysis should be used as far as possible. When, as is at present the case with potash, there is a fertilizer shortage, this is particularly important.

Introduction

The changing needs and conditions of war demand a flexible fertilizer policy, which must be directed towards the maximum production of the most necessary crops, and at the same time ensure every possible economy in the use of fertilizers themselves.

Both potash and phosphate have to be imported. Potash will in any event be in short supply, and additional supplies of phosphate can be justified only if they effect more than a corresponding economy in the imports of food.

Farmers, therefore, have to support their claims for increased supplies of fertilizers by evidence of increases in production. Such evidence must be quantitative, and not merely qualitative. Equally they are vitally concerned in using fertilizers to the best advantage, not only in the national interests but also in their own interests—for fertilizers cost money and the amounts available are limited.

To use fertilizers to best advantage demands accurate knowledge of their effects, and this can only be obtained from experiments. It is now well known that the responses to fertilizers are very variable, depending on crop, soil, season, &c. Consequently final conclusions must never be based on the results of a single experiment, or even a series of experiments on a single farm, but on a large number of experiments on different farms, conducted in different years, and on different crops.

Once the results of a large number of experiments are available the average responses to the various fertilizers can be determined. Such averages, in so far as they refer only to broad districts and cover many soil types in the district, must, of course, be interpreted in the light of local knowledge and experience, but they provide a sound working basis for assessing the general needs of the various crops. With a sufficiently accurate and extensive series of experiments, averages for particular districts and soil types can also be obtained. Moreover, if soil analyses have been conducted on the experimental land, the relationship between the actual responses and the values given by soil analysis can be studied. In this country, however, there is not yet a sufficient body of data for this to be possible. Indeed only on sugar-beet and potatoes has any progress been made in testing the association between responses and the results of soil analysis.

In order to obtain an accurate picture of the average effects of the various fertilizer components, and so enable a sound fertilizer policy to be drawn up, all fertilizer experiments conducted in Great Britain since 1900,¹ and similar experiments in certain other northern European countries, have recently been brought together and summarized. The present paper describes the results of this inquiry, and shows how they affect the fertilizer policy of the farmer.

It is perhaps somewhat remarkable that such a summary has never previously been made. Probably the main cause of this is the heterogeneity of the experiments and the inaccessibility of the results. It is much to be deplored that no comprehensive series of experiments on varying levels and combinations of the three standard fertilizers has yet been undertaken in this country for any crop except sugar-beet. Even on the Continent little has been done in this direction, but there are far greater numbers of simpler experiments. The British experiments have, however, given consistent results and these results find strong confirmation from the parallel continental experiments.

The results in the present inquiry are restricted to those of single-year

¹ This date was chosen since changes in methods of farming and consequent variations in soil fertility are likely to make the results of earlier experiments progressively less applicable. In particular, during the last few decades of the nineteenth century there was a large increase in the use of artificial fertilizers.

experiments, and, in consequence, underestimate the total effect of fertilizers by omitting the residual or cumulative effects. But under war-time conditions the immediate response by the crop to which the fertilizer is supplied is of first importance, and evidence is growing to suggest that there is a general tendency among farmers to exaggerate the value of residual effects of fertilizers used in good crop rotations, since highly responsive crops which are heavily manured are normally followed by less responsive crops. Thus phosphates may make all the difference between the success and failure of a root crop and yet have negligible effects on the following cereal crop.

Although in some respects the results of the inquiry merely confirm current beliefs and practice, they also reveal some unexpected facts and strongly support the contentions that have already been made in certain quarters that the main war-time fertilizer policy of this country should be to encourage the use of more nitrogenous fertilizers. This will result in more profit to the farmer and more food for the nation even should the supplies of phosphate and potash be relatively curtailed.

In summarizing the experiments the responses given with different levels of dressing have been reduced to responses to a standard dressing by means of the response curves discussed later. The standard dressings actually chosen were:

Sulphate of ammonia, 1·2 cwt. per acre (0·25 cwt. N.).

Superphosphate, 3 cwt. per acre (0·5 cwt. P_2O_5).

Sulphate or muriate of potash, 1 cwt. per acre (0·5 cwt. K_2O).

These values were chosen as being about equal to the average dressings used in the experiments themselves. They are of course low for the root crops.

In the Danish and East Prussian experiments this adjustment could not be made, since in the original reports it was assumed that the responses were proportional to the amounts of fertilizer over the range of dressings normal for each crop.

Average responses.—The average responses shown by the various crops are given in Tables 1 and 2. Each value is accompanied by the number of experiments from which it was derived. For roots the experiments in the British Isles were subdivided into those with and without dung. For cereals only those without dung are included. This subdivision was not possible for the continental experiments, but the majority of the root crops were grown with, and cereals without, dung.

In the original analysis Great Britain was divided into eight districts,¹ but for economy of presentation the results from districts showing similar responses have been combined. Thus one set of values is shown for nitrogen, three for phosphate, and three for potash.

The mean values for Great Britain are simple averages of all experiments, but the general mean was obtained by weighting the averages for each country by the number of experiments, with an upper limit of 200,

¹ The districts originally chosen for England were as follows: *South-west*: Cornwall, Devon; *West Midlands*: Somerset, Wiltshire, Gloucester, Worcester, Warwick, Hereford, Shropshire, Stafford, Cheshire, Lancashire; *North*: Yorkshire, Westmorland, Durham, Cumberland, Northumberland; *South and East*: all other counties.

TABLE I. *Response of Root Crops to Fertilizer Treatment*

	Mean response (tons/acre)				Number of experiments			
	Swedes	Man- golds	Sugar- beet	Pota- toes	Swedes	Man- golds	Sugar- beet	Pota- toes
NITROGENOUS FERTILIZER (0.25 cwt. N per acre)								
<i>With dung:</i>								
Great Britain	2.3	2.6	0.92	0.86	186	183	56	284
Ireland	1.4	2.7	..	1.42	268	286	..	60
Denmark	2.2	2.6	1.19	0.62	3,198	3,494	440	1,153
South Sweden	2.6	3.0	1.46	0.84	267	404	1,452	404
East Prussia	0.90	728
General mean	2.1	2.8	1.28	0.85	3,919	4,367	1,948	2,629
<i>Without dung:</i>								
Great Britain	2.2	3.1	0.88	1.07	330	98	227	212
Ireland	2.2	162
PHOSPHATIC FERTILIZER (0.50 cwt. P ₂ O ₅ per acre)								
<i>With dung:</i>								
S. and E. England . . .	2.0	0.5	0.84	0.36	13	82	21	68
W. Mid. and N. England .	1.3	1.3	?	0.26	84	99	8	104
SW. England, Wales, and Scotland	3.5	1.4	?	1.18	84	27	1	75
Great Britain	2.3	1.0	0.66	0.55	181	208	30	247
Ireland	4.3	1.8	..	0.84	387	206	..	449
Denmark	1.8	1.1	0.76	0.54	2,774	3,011	434	844
South Sweden	1.6	1.7	0.45	0.49	247	417	1,557	355
East Prussia	0.64	725
General mean	2.5	1.4	0.61	0.61	3,589	3,842	2,021	2,620
<i>Without dung:</i>								
S. and E. England . . .	2.5	0.7	0.42	0.93	78	22	147	80
W. Mid. and N. England .	3.7	1.6	0.38	0.66	65	30	34	105
SW. England, Wales, and Scotland	5.9	4.9	?	1.48	133	24	3	18
Great Britain	4.4	2.4	0.41	0.84	276	76	184	203
Ireland	10.8	4.15	313	14
POTASSIC FERTILIZER (0.50 cwt. K ₂ O per acre)								
<i>With dung:</i>								
S. England	1.3	1.2	0.11	0.45	19	104	27	103
W. Mid., N. England, Wales Scotland	0.5	2.3	0.28	0.34	100	102	11	136
Scotland	1.8	..	?	1.32	62	..	1	66
Great Britain	0.9	1.7	0.17	0.55	181	206	39	305
Ireland	1.3	3.0	..	0.91	62	236	..	412
Denmark	0.5	1.0	0.28	0.47	3,425	3,518	451	1,189
South Sweden	0.7	1.4	0.30	0.46	253	420	1,557	347
East Prussia	0.21	705
General mean	0.9	1.8	0.29	0.52	4,121	4,380	2,047	2,958
<i>Without dung:</i>								
S. England	0.7	1.8	0.42	1.04	89	52	165	127
W. Mid., N. England, Wales Scotland	2.3	2.3	0.50	1.38	89	28	46	123
Scotland	3.1	..	?	1.96	191	..	8	12
Great Britain	2.3	1.9	0.44	1.23	369	80	219	262
Ireland	3.4	148

a procedure which prevents undue weight being given to countries with very large numbers of experiments. The means of groups of less than ten experiments are not shown in the Tables.

TABLE 2. *Response of Cereal Crops to Fertilizer Treatment*

	Responses in cwt. grain per acre			Number of experiments		
	Wheat	Barley	Oats	Wheat	Barley	Oats
NITROGENOUS FERTILIZER (0.25 cwt. N per acre)						
<i>Without dung:</i>						
Great Britain	3.4	3.7	3.4	100	61	86
Ireland	4.4	..	2.7	135	..	180
Denmark	4.2	3.8	3.6	381	3,312	2,275
South Sweden	3.7	4.9	4.2	240	241	122
East Prussia	3.2	3.8	4.2	171	211	313
General mean	3.8	4.1	3.6	1,027	3,825	2,976
PHOSPHATIC FERTILIZER (0.5 cwt. P ₂ O ₅ per acre)						
<i>Without dung:</i>						
S. and E. England . . .	?	0.4	?	5	57	7
W. Mid., N. England . .	?	0.8	2.2	9	25	22
S.W. England, Wales, Scotland	..	?	1.2	..	9	31
Great Britain	0.3	0.6	1.5	14	91	60
Ireland	2.8	150
Denmark	0.6	1.8	1.7	162	3,334	2,108
South Sweden	0.4	0.9	1.4	201	223	141
East Prussia	1.9	1.8	2.3	168	205	304
General mean	1.0	1.4	2.0	545	3,853	2,763
POTASSIC FERTILIZER (0.5 cwt. K ₂ O per acre)						
<i>Without dung:</i>						
S. England	?	0.4	0.8	3	49	13
W. Mid., N. England, Wales .	?	0.4	0.1	9	25	12
Scotland	?	1.0	..	8	30
Great Britain	2.3	0.4	0.8	12	82	55
Ireland	1.3	150
Denmark	0.5	0.8	0.5	128	3,734	2,559
South Sweden	0.4	0.4	1.0	200	223	142
East Prussia	0.7	0.8	1.0	169	206	308
General mean	0.6	0.6	0.9	509	4,245	3,214

All but a small proportion of the experiments were conducted on ordinary commercial farms, and examination of the differences between different series of experiments suggests that there is little bias through the selection of abnormally rich or poor soils.

For phosphate and potash, but not for nitrogen, there was evidence that the responses to fertilizer fell off as the basic fertility of the soil

increased. As might well be expected poorer soils tended to give greater responses. Phosphate and potash behave differently from nitrogen, partly because they are retained in the soil to build up reserves, and partly because these reserves provide particularly favourable conditions for responses to nitrogen.

In most of the experiments each fertilizer was tested in presence of moderate amounts of the other principal fertilizers. The more accurate and comprehensive modern experiments also give information on the effects of each fertilizer on the responses to the others.

The continental experiments differ from the British in that the majority of experiments on crops other than potatoes were made with nitrate fertilizers, those on sugar-beet and mangolds with Chile nitrate of soda, and the others mainly with synthetic nitrate of lime. Nitrates, especially nitrate of soda, usually give better responses per unit nitrogen than sulphate of ammonia, but the difference is to a large extent offset by the extra cost of unit nitrate nitrogen. The continental experiments on potassic fertilizers were made almost exclusively with 40 per cent. potash salt.

A close study of the responses given in Tables 1 and 2 shows that whereas, as might be expected, there are certain irregularities, the values on the whole are very consistent. The agreement between the means for various crops in Great Britain and the general means are remarkably close. To some extent, however, this must be regarded as fortuitous, particularly for the case of phosphate, where there are large variations between different parts of Great Britain.

Influence of dung.—Leaving aside for the moment the differences between crops, the most remarkable feature of the results is the small difference between the responses to nitrogen in the absence of dung and in the presence of dung. One reason which might account for this is that the experiments with dung were conducted on more exhausted land (which was dunged just because it was more exhausted) with the consequence that they responded better to nitrogen because their needs were greater. In order to obtain more definite information on this point, which is of considerable theoretical and practical importance, certain series of experiments which tested the response to nitrogen both in the presence and the absence of dung were abstracted separately. These comparisons confirmed the general conclusion that dung has little effect on the response to nitrogenous fertilizers.

The responses to phosphate and potash on the other hand are substantially reduced in the presence of dung, and from the values given in Table 1 the general reduction for each of these fertilizers may be roughly assessed at 50 per cent. Even these reductions are somewhat smaller than might be expected from the average nutrient contents of dung as shown by chemical analysis. A similar examination of experiments which tested the responses to phosphate and potash both in the presence and absence of dung, gives somewhat greater reductions, particularly in the case of potash.

The explanation of these facts would appear to be as follows:

Dung is a source of the three nutrients considered, as well as of others less generally important. In addition, it improves the physical condition

of the soil. The aggregate effect of the improvement by dung, and the fact that it is normally used on the more exhausted soils, render the crops particularly responsive to the nutrients supplied by added fertilizers. Since most soils are deficient in available nitrogen the response to nitrogenous fertilizers is high even in the presence of the available nitrogen supplied by the dung. The exhaustion of phosphate and potash, which are retained in the soil, is normally less drastic, and the effects of added phosphatic and potassic fertilizers are reduced through the amounts of these nutrients supplied by the dung; the reduction is, however, less than would be expected from the actual amounts in the dung, owing largely to the previous partial exhaustion of the soil.

Practical recommendations for the manuring of dunged and undunged land can reasonably be based on the values of the average responses given in Table 1. This will make automatic allowance for the fact that dung tends to be used on somewhat poorer land.

The average responses to dung in the British experiments were extracted in the same manner as for fertilizers and reduced to a standard dressing of 10 tons per acre. The results are shown in Table 3 for crops grown without fertilizers and with fertilizers respectively.

TABLE 3. *Crop Responses to 10 Tons of Dung per acre*

	<i>Responses in tons per acre</i>			<i>Number of experiments</i>		
	<i>Swedes</i>	<i>Mangolds</i>	<i>Potatoes</i>	<i>Swedes</i>	<i>Mangolds</i>	<i>Potatoes</i>
<i>Fertilizers absent:</i>						
S. and E. England . . .	4.6	4.2	2.0	43	13	41
W. Mid. and N. Eng- land	6.1	5.5	3.8	72	18	121
SW. England, Wales, and Scotland . . .	7.1	9.4	3.4	78	51	72
Great Britain	6.4	7.7	2.8	193	82	234
<i>Fertilizers present:</i>						
Great Britain	2.6	2.7	1.4	84	21	132

The average responses are large, especially in the wetter regions and where no artificials are used. Potatoes, mangolds, and swedes show similar percentage responses. It is clear that if supplies of dung are limited, potatoes should have preference, since the financial returns from the increases in yield of potatoes are relatively much greater than from the root crops, particularly when artificial fertilizers are also used.

Regional variation in response.—The responses to nitrogen show very little variation, and a single set of mean responses has therefore been shown. Potash does show some variation, the responses in general being greater in the north than in the south. The differences are, however, not sufficiently large or well established to be taken into account, and in the subsequent discussion of optimal dressings single mean values for Great Britain have been taken for the whole of the country.

The situation with regard to phosphate is entirely different. Here there are marked variations in response; the wetter regions show higher

responses than the drier south and east of England. These differences have therefore been taken into account in the subsequent discussion.

These differences in phosphate-response are of course well known. The effect of increasing rainfall on the response to the phosphate may act directly through its effect on crop-growth and the length of the growing-season, and it may act indirectly through the soil. Most soils in wet districts are acid because lime is readily lost by drainage, and acid soils stand in particular need of phosphatic fertilizers. This is illustrated in Table 4, which shows how the response of sugar-beet to the standard dressing of superphosphate varied with the reaction of the soil in some experiments in South Sweden.

TABLE 4. *Response of Sugar-beet to Superphosphate in South Sweden*

<i>Soil reaction</i>	<i>pH</i>	<i>Tons sugar-beet roots per 3 cwt. superphosphate</i>	<i>Number of experiments</i>
Acid . . .	below 6.5	1.03	180
Slightly acid . . .	6.7 to 7.0	0.68	169
Slightly alkaline .	over 7.0	0.33	169

In wet regions and on acid soils phosphatic manuring is obviously of the utmost importance. At the present time the need will be especially great on the newly broken-up grass-land of the wetter regions. The need for lime as well naturally remains, for liming acid soils not only increases crop yields but also allows phosphates to remain available much longer. The greater effect of dung in the wetter regions is also to be ascribed largely to the phosphate and potash it supplies.

Relative responses of different crops.—Tables 1 and 2 may now be condensed. In this condensation it has been assumed that the ratio of the responses of the different crops is similar in all districts. This appears to be broadly true, except possibly for swedes, which are not a particularly suitable crop for the south and east of England and appear to show rather smaller responses in this district.

Contrary to the general belief, there appear to be no great differences between the responses of the different cereal crops, and they have consequently been grouped together. Wheat gave a lower average response than oats to phosphate, and in the continental experiments rye proved the most responsive of the cereals to phosphate. These differences are almost certainly accounted for by the tendency to grow wheat on soils and in regions which have low phosphate-requirements, whereas rye is normally grown on poor acid sands.

The summary of the various responses to the standard dressings is shown in Table 5, where the mean yields and values of the various crops, and their starch and protein equivalents are also shown. The prices of swedes and mangolds have been raised somewhat above conventional estimates in view of the scarcity of imported feeding-stuffs. An allowance was also made for the effects of fertilizers on sugar-beet tops, which were valued at 14s. per ton. The price of cereal grain was increased to 15s. per cwt. to make some allowance for the additional straw.

TABLE 5. *Average Responses to Standard Dressings of Fertilizers for Root Crops grown with, and Cereals grown without, Dung**Standard dressings per acre*

0.25 cwt. N = 1.2 cwt. sulphate of ammonia.
 0.50 „ P_2O_5 = 3.0 „ superphosphate.
 0.50 „ K_2O = 1.0 „ muriate or sulphate of potash.

Note. For roots grown without dung the responses given should be increased by 10 per cent. for nitrogenous manures and by 100 per cent. for potassic and phosphatic manures.

<i>Response in tons roots or cwt. grain</i>	<i>Swedes</i>	<i>Mangolds</i>	<i>Sugar-beet</i>	<i>Potatoes</i>	<i>Cereals</i>
<i>Sulphate of ammonia</i> . . .	2.1	2.7	0.9	0.9	3.5
<i>Superphosphate</i>					
S. and E. England . . .	1.2	0.7	0.3	0.3	0.7
W. Mid. and N. England . .	1.8	1.0	0.4	0.5	1.0
SW. England, Wales, Scotland . . .	3.0	1.7	0.7	0.8	1.7
<i>Sulphate or muriate of potash</i> . .	1.0	1.6	0.25	0.55	0.7
Mean yields with fertilizer . . .	20	25	11	9	21
Value per ton roots or cwt. grain .	23s.	19s.	60s.	110s.	13s. 6d.
Starch equivalent, per cent. . .	7.3	6.5	15.0	17.8	67.5
Protein equivalent, per cent. . .	0.7	0.4	0.6	0.8	8.1

Nitrogen.—Potatoes, mangolds, and swedes show average responses of the order of 12 per cent. Sugar-beet is less responsive in roots (about 9 per cent.) but owing to its cash value, its high energy-content, and the additional response of about 1.3 tons of tops for the standard dressing, the total return in starch equivalent is the highest of all crops, and the cash return at present prices is second only to that from potatoes.

Grain crops show average responses of the order of 20 per cent. to 1.2 cwt. of sulphate of ammonia or its equivalent. Contrary to a common opinion, the large number of experiments here and abroad show little or no clear differences between the four cereals, wheat, barley, oats, and rye, in their responses to nitrogenous fertilizers.

Phosphate.—Swedes showed much greater percentage responses to phosphate than any other crop, the responses in the absence of dung ranging from about 20 per cent. in south and east England to 50 per cent. in the north of Scotland. Potatoes and mangolds showed about half the percentage responses of swedes. Sugar-beet showed still smaller responses.

The cereals, too, showed only small percentage responses to phosphate. There is thus abundant justification for the normal practice of restricting phosphatic manures to the root and ley breaks of the rotation, but this conclusion may need modification for two sets of conditions not covered in the experiments. Phosphate may be needed (1) for cereals undersown with seeds because it improves the 'take' of the clovers, and (2) for cereals on newly ploughed-out poor and acid grassland. The latter qualification is, however, contained in the general one that the data refer to average soils and conditions, and that for individual fields

allowance must be made for the soil's nutrient-content as shown by soil analysis, or as estimated by local knowledge and experience.

On the basis of present prices the order of responsiveness to phosphates is potatoes, swedes, mangolds, sugar-beet, cereals. On the basis of starch equivalent, swedes take first place and potatoes second.

Potash.—Potatoes are the most responsive crop, 1 cwt. of sulphate of potash increasing the crop by 18 per cent. in the absence of dung and by 6 per cent. in the presence of dung. Swedes and mangolds are somewhat less responsive and sugar-beet gives only a small percentage response in roots, total sugar, and tops. Potash increased the sugar percentage by a small fraction of 1 per cent., but the corresponding cash return was small.

On the basis of both prices and starch equivalent the order of responsiveness is potatoes, swedes, mangolds, sugar-beet, cereals.

Salt.—Agricultural salt, of which, by complete contrast with potash, this country has vast deposits, has given good responses on both mangolds and sugar-beet, especially when neither dung nor potash was used. The responses of mangolds to 4 cwt. salt were as follows:

	<i>Without dung</i>		<i>With dung</i>	
	<i>Without potash</i>	<i>With potash</i>	<i>Without potash</i>	<i>With potash</i>
Response (tons) .	3.4	2.7	2.7	1.8
No. of experiments .	33	24	79	58

In 1940 a series of 24 sugar-beet experiments on representative soils gave the following average yields in tests on 2½ cwt. muriate of potash and/or 5 cwt. of salt per acre, in the presence of a basal dressing of 4 cwt. of sulphate of ammonia.

	<i>Washed roots, tons per acre</i>		<i>Total sugar, cwt. per acre</i>	
	<i>No potash</i>	<i>Potash</i>	<i>No potash</i>	<i>Potash</i>
No salt . . .	11.3	12.1	42.3	45.7
Salt . . .	12.5	12.6	47.1	47.6

Used separately salt and potash gave good responses, the response to salt being slightly greater than that to potash. In the presence of salt, potash had a negligible effect, but in the presence of potash, salt still gave a profitable return.

Dressings of from 3 to 5 cwt. salt per acre are suitable and should be applied some time before sowing to reduce risk of delayed germination. The value of the sodium chloride in 30 per cent. potash-salt explains why this form was recommended in the past for sugar-beet and mangolds.

Interactions.—In the previous sections the responses to the different artificial fertilizers have been discussed separately, with the implied assumption that their effects are independent. In reality the situation is more complicated, the response to each fertilizer being influenced to a greater or less extent by the level of the other fertilizers. Such effects are called interactions.

Interactions are of two kinds:

(a) One fertilizer may enhance the effect of another (positive interaction), as commonly happens with radically different fertilizers, such as nitrogen, phosphate, and potash.

(b) One fertilizer may reduce the effect of the other (negative interaction), as commonly happens when both fertilizers provide the same plant-nutrient. The interactions between dung and phosphate and between dung and potash are illustrations of negative interactions. Occasionally negative interactions occur between radically different fertilizers, but such effects are rare.

Unfortunately most of the experiments under review furnish no clear estimates of these interactions, but a sufficient number of modern experiments on potatoes and sugar-beet have now been completed to show that the interaction effects are somewhat complicated, and also vary from crop to crop. With sugar-beet there is a definite positive interaction between nitrogen and potash, and some indication of a similar but smaller interaction between nitrogen and phosphate. There is little interaction between potash and phosphate. With potatoes there are positive interactions between nitrogen and phosphate, and between phosphate and potash, but there is little interaction between nitrogen and potash.

Like the responses to the different fertilizers, the interactions vary considerably from experiment to experiment, being in general larger when the responses themselves are large. As a general rule it may be taken that there will be little interaction involving any fertilizer which does not itself produce a good response. This is, of course, what might be expected. On general grounds a crop is likely to respond less well to any nutrient component if the other nutrient components are inadequately supplied.

The existence of interactions is commonly used as an argument in favour of properly balanced mixtures or compound fertilizers, adjusted to the needs of individual crops, but applied regardless of soil. The point that is overlooked by the advocates of the uncritical use of such mixtures is that the soil may itself furnish adequate supplies of one or more of the nutrients, in which case there will only be small direct responses to these nutrients, and equally there will be little interaction involving them. The main practical merit of these compound fertilizers lies in the fact that they supply in a convenient form some of each of the three leading plant-foods, and thus cover the risk that any one of these foods may be more deficient than the others. Any widely used compound manure caters for average soils over a wide range of conditions, and to use it is to assume that one's own soil has no special peculiarities which could be met more economically.

With increasing use of the results of field experiments and soil analysis it will be possible to adjust the amounts of phosphate and potash fertilizers more closely to the needs of individual farms and fields. Under peace conditions it was perhaps justifiable to pay somewhat heavily for covering the risks of unsuspected shortages in the hope of building up reserves of plant-nutrients in the soil, but under war conditions it is most

important to adapt manuring much more closely to individual soils, and to 'cash in' on such reserves as do exist. Some fertilizers which are in short supply are necessarily expensive, and it is wasteful for both farmer and nation to use them where they are not in fact needed. It would have been foolish, for example, as is shown in the discussion on optimal dressings, to spread the restricted supply of potash over a wide area of land by a proportionate reduction of the potash-content of all the usual compound fertilizers. Instead, potash has been restricted to certain priority crops, notably potatoes, and to soils known to be deficient in potash. Compound manures for other crops and soils are thus rightly

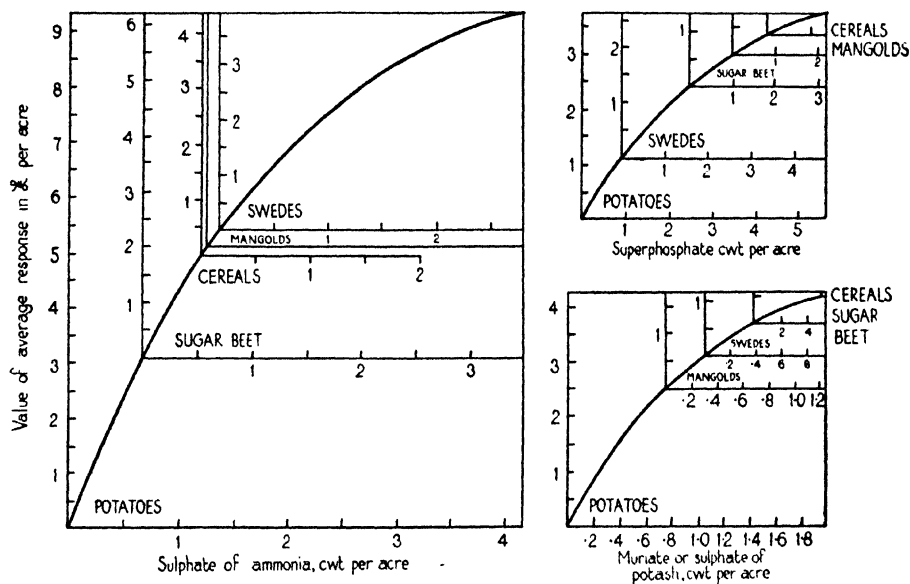


FIG. 1. Value in £ per acre at 1940-1 prices of average responses to fertilizers for root crops grown with dung, and cereals without dung.

(The effects of superphosphate are shown for the West Midlands and North England.)

unbalanced. Similarly, on any but definitely phosphate-deficient soils, it is unnecessary to use phosphates in mixtures for cereals: indeed, most cereals should receive nothing beyond a nitrogenous fertilizer.

Optimal dressings.—When considering how much fertilizer to apply to a crop, the variation in response with varying amounts of the fertilizer must be taken into account. The response to unit weight of any fertilizer falls off as the total amount of the dressing is increased. The exact form of the curve relating amount of dressing and response varies with varying circumstances, but for the purpose of this inquiry it is sufficient to take the standard form described in the Appendix. The curve is illustrated in Fig. 1, which will be explained later.

The most efficient or 'optimal' dressing will be that which gives the maximum financial return after allowing for the cost of the fertilizer and any additional costs resulting from the larger crop. This optimal level

will be reached when the value of the increase in crop resulting from a further small additional dressing is exactly equal to the cost of this additional dressing.

As an example we may consider the optimal dressing of sulphate of ammonia for potatoes grown with dung.¹ The average response to the standard dressing (Table 5) is 0.9 tons. Taking the cost of the standard dressing as 15s. per acre and the value of the crop as 110s. per ton, we obtain responses and costs as in Table 6:

TABLE 6. *Cash Return from the Use of Sulphate of Ammonia for Potatoes (per acre)*

<i>Dressings of sulphate of ammonia</i>		<i>Responses</i>		<i>Net return</i>
<i>Cwt.</i>	<i>Cost</i>	<i>Tons</i>	<i>Value</i>	
1.2 (standard)	15s.	0.90	99s.	84s.
2	25s.	1.26	139s.	114s.
3	38s.	1.53	168s.	130s.
4	50s.	1.69	186s.	136s.
5	62s.	1.78	196s.	134s.
6	75s.	1.84	202s.	127s.
7	88s.	1.87	206s.	118s.

The optimal dressing is about 4 cwt. of sulphate of ammonia per acre, but over a wide range around the optimal (from 3 to 6 cwt. per acre) the net profit changes but slightly. Dressings somewhat below the theoretical average optimum will normally be the most attractive to farmers, for the net return will approach the maximum with a lower total outlay than would be required to manure up to the average optimum. But although the profits to the farmer remain fairly steady over a wide range around the optimal, the total production continues to be increased appreciably by manuring to levels well above the optimum.

The dressings which are optimal to the individual farmer will only be optimal from the point of view of the nation if the relative prices of fertilizers and crops accurately represent their relative values to the nation. In deciding these questions it is necessary to consider the alternatives, in terms of costs and shipping facilities, of importing additional food or fertilizers.

Table 7 gives the calculated optimal dressings at 1940-1 prices of crops and fertilizers² for root crops grown with and without dung and for cereals without dung.

¹ The method of calculating the optimal dressing directly is described in the Appendix. In this example the value/cost ratio (v/c) is $0.9 \times 110/15 = 6.6$, and from Table 12 the corresponding optimal dressing is 0.86 cwt. N, or 4.1 cwt. sulphate of ammonia. The value of the increase in crop is $2.13 \times 99 - 1.58 \times 15 = 187$ shillings, and the net return is $187 - 0.86 \times 60 = 136$ shillings.

² For simplicity 15s. has been taken as the cost of each standard dressing, representing values intermediate between sulphate of ammonia and nitro-chalk, superphosphate and basic slag, and muriate and sulphate of potash, with some allowance for cost of application.

TABLE 7. *Optimal Dressings of Fertilizers*

N = Nitrogenous fertilizer expressed as cwt. sulphate of ammonia per acre.

P = Phosphatic fertilizer expressed as cwt. superphosphate per acre.

K = Potassic fertilizer expressed as cwt. sulphate or muriate of potash per acre.

Optimal dressings

	<i>Great Britain</i>	<i>S. and E. England</i>	<i>W. Midlands and N. England</i>	<i>S.W. England, Wales, and Scotland</i>	<i>Great Britain</i>
<i>With dung:</i>	N	P	P	P	K
Swedes . . .	2.7	3.5	4.8	6.5	0.9
Mangolds . . .	2.9	0.8	2.2	3.8	1.2
Sugar-beet . . .	3.5	1.9	3.2	4.9	0.6
Potatoes . . .	4.1	4.3	5.6	7.3	2.0
<i>Without dung:</i>					
Swedes . . .	2.9	5.8	7.1	8.8	1.7
Mangolds . . .	3.0	3.1	4.4	6.1	2.0
Sugar-beet . . .	3.7	4.1	5.4	7.1	1.3
Potatoes . . .	4.3	6.6	7.9	9.6	2.7
Cereals . . .	2.0*	0.1	1.4	3.1	0.1

Average crop responses for above dressings when used without dung

	Roots: tons per acre				
Swedes . . .	3.9	3.3	5.3	9.3	2.6
Mangolds . . .	5.1	1.4	2.5	4.7	4.5
Sugar-beet . . .	1.8	0.6	1.1	2.0	0.6
Potatoes . . .	1.9	1.0	1.5	2.7	1.7
	Grain: cwt. per acre				
Cereals . . .	4.8	0.1	0.6	1.7	0.1

* Arbitrary upper limit.

Many of the optima may seem unduly high, but it is interesting to note that before the war the consumption of nitrogenous fertilizer per acre of arable land was over five times as high in Holland and Belgium and well over twice as high in Germany as in Great Britain.

The most serious discrepancy between optimal and current average rates of manuring is for nitrogen on cereals. From the average responses already given, the calculated optimal dressing amounts to 3.0 cwt. sulphate of ammonia per acre. This quantity might often cause lodging, and an upper limit of 2 cwt. sulphate of ammonia per acre for cereals has therefore been adopted in subsequent discussion. By manuring to this average rate it is estimated that there would be a gross return from the cereals grown on pre-war acreages of about £17,000,000, for nitrogenous fertilizers costing about £6,000,000. If an average of only 1 cwt. sulphate of ammonia per acre for cereals were attained, it would give a return on pre-war acreages of £11,000,000 for an outlay on fertilizer of £3,000,000. In pre-war practice the return was about £4,000,000 for an outlay of about £1,000,000. In a survey of a random selection of farms a few years ago it was found that two-thirds of the farmers failed to give any fertilizer to the grain crops preceding their potatoes.

This discrepancy between the highly consistent results of actual trials in Great Britain, Ireland, and three Baltic countries, and the current practice of British farmers calls for explanation. Fear of lodging is undoubtedly one of the causes. Another probable cause is that farmers have failed to appreciate the fact that under normal conditions cereals require only a nitrogenous fertilizer, applied in the spring either in the seed-bed or as a top-dressing. They may have tried compound cereal manures and been disappointed, without realizing that little return was to be expected from the phosphate and potash. Barley-growers have feared that the nitrogenous fertilizer would reduce the malting quality of their barley, but many experiments have shown that up to 1 cwt. of sulphate of ammonia per acre may safely be used without perceptible effect on malting quality, and even more under favourable conditions. Finally, one of the main factors has been the simple fact that reliable cereal experiments are difficult to conduct and have not therefore been widely attempted in this country.

For roots also the optimal dressings require a greater proportion of nitrogen than is generally used. Thus for potatoes grown with dung in the West Midlands and North England the optimal dressings, as given in Table 7, are 4.1 cwt. sulphate of ammonia, 5.6 cwt. superphosphate, and 2.0 cwt. sulphate of potash per acre, or 0.86 cwt. N; 0.93 cwt. P_2O_5 ; 1.0 cwt. K_2O . In a random sample of 500 potato farms in 1937 the average amounts of fertilizer in cwt. per acre were 0.36 N, 0.52 soluble P_2O_5 , 0.11 insoluble P_2O_5 , 0.68 K_2O .

Subject to the general condition that the south and east of England need less and Scotland and Wales more phosphate than the average, it may be said that the optimal requirements for root crops grown with dung are roughly equal amounts of nitrogen, phosphoric acid, and potash, or in a compound fertilizer about equal percentages of N, soluble P_2O_5 , and K_2O . Relative to the amount of nitrogen used, swedes require more phosphate, and mangolds and sugar-beet less; sugar-beet also needs less potash. For rates of manuring below the optimal the proportion of nitrogen to the other constituents should be still further increased.

The older types of compound fertilizer generally provide a much greater percentage of phosphoric acid than of nitrogen. In the last fifteen years or so there has been a general tendency to recommend higher proportions of nitrogen and potash in potato mixtures, as a result of experiments, first at Kirton and then at various centres by the Rothamsted staff, which showed that many potato-growers were using uneconomically high amounts of phosphate.

In the aggregate the pre-war consumption of superphosphate on arable land was a reasonable fraction—around two-thirds—of the estimated optima for the acreage, but there can be little doubt that its distribution among crops and between soils was far from ideal. Some of the reserves built up by heavy phosphatic manuring in the past can be utilized in war-time on certain classes of soils, but it must be remembered a large fraction of the phosphate added to soils is unavoidably lost by its conversion into unavailable forms, especially on acid soils. In this

connexion it must also be remembered that not only will newly ploughed-out grassland on acid soils need lime and phosphate to fit it for arable crops, but that the phosphatic dressings will have to be repeated more frequently than would be customary for arable land in good heart.

The outstanding need for potash is clearly that of potatoes. We have estimated that even at the actual current rates of application about 70 per cent. of the total return for potash among the field crops here considered comes from potatoes. The immediate problem is not to increase the dressings to the optimal but to make the best use of a decreasing supply. For this purpose it is essential that a still larger proportion of the total available potash should find its way to the most responsive crop—the potato—and not be disseminated over crops indiscriminately through an undue belief in the virtues of balanced fertilizers.

Effects of changing supplies.—At the present time both the country as a whole and the individual farmers are faced with the problem of adjusting fertilizer allocation among various crops and among different districts or fields in such a way as to secure the biggest output from changing supplies. Potash supplies are already short; other fertilizers manufactured in this country and formerly exported may be available in larger quantities, though occasions may arise either in districts or on individual farms when farmers cannot obtain all they require. It is necessary therefore to have some sound key to the problem of making adjustments, rapidly if need be.

The maximum financial returns from a fertilizer on an average farm or throughout the country will be greatest when each crop receives its optimal dressing. With smaller supplies of a fertilizer the greatest returns will be obtained when the total amount on a farm or throughout the country is distributed so that the *dressings for all crops are reduced by the same amount below their optimal dressings*. The reduction should not be a proportionate one.

By subtracting suitable constant amounts from the values for the optimal dressings given in Table 7, a series of rates for the different crops can be obtained, and these can be used in conjunction with the acreages of the crops to determine the best distribution of a fertilizer on a farm. In the case of superphosphate, for example, the best allocation among crops at different levels of total supply can be obtained by trial from the figures set out in Table 8 (where reductions by steps of 1.5 cwt. per acre are made), multiplying the acreages of the various crops by their requirements. The last column shows the results of such a calculation for a farm with the acreages shown in the last line of the table.

If the farmer wishes to allocate 10 tons (200 cwt.) of superphosphate, he should apply amounts of fertilizer intermediate between those given in lines 3 and 4 of the table. As the theoretical dressing for mangolds is very small, no superphosphate need in practice be applied to this crop or to the cereals. The allocation between crops might therefore be as follows: swedes, 5 cwt. per acre; sugar-beet, $3\frac{1}{2}$ cwt. per acre; potatoes, 4 cwt. per acre.

Such an allocation is, in fact, the common practice of many farmers at the present time, for they give most or perhaps the whole of their phos-

phate and potash to the root crops, especially swedes and potatoes, and restrict the manuring (if any) of their cereals to a nitrogenous top-dressing.

TABLE 8. *Optimal Requirements of Superphosphate in cwt. per acre at Varying Levels of Total Supply, with an Example for a Single Farm*

O = without dung. D = with dung.

Optimal dressings for:	Swedes		Mangolds		Sugar-beet		Potatoes		Cereals	Example: Total Superphosphate required (cwt.)
	O	D	O	D	O	D	O	D	O	
Scotland, &c.	8.8	6.5	6.1	3.8	7.1	4.9	9.6	7.3	3.1	693
W. Midlands, &c.	7.3	5.0	4.6	2.3	5.6	3.4	8.1	5.8	1.6	468
S. and E. England	5.8	3.5	3.1	0.8	4.1	1.9	6.6	4.3	0.1	243
	4.3	2.0	1.6	..	2.6	0.4	5.1	2.8	..	151
	2.8	0.5	0.1	..	1.1	..	3.6	1.3	..	61
Example: acreage of crops on farm	10	10	20	20	90	

These relationships between the different crops are illustrated in Fig. 1, which shows the response-curves for roots with and cereals without dung, arranged so that equally efficient dressings are vertically above one another. The extreme right of the diagram shows the optimal dressings. From these curves the average response for any dressing can be read off. Equal reductions below optimal dressings cause equal reductions below optimal returns, because the response-curves for various crops are in fact part of the same curve, once crop and fertilizer prices have been fixed.

TABLE 9. *Fertilizer Mixtures for Potatoes at Varying Costs (W. Midlands and N. England)*

Cost of fertilizer mixture per acre	With dung			Without dung		
	N	P ₂ O ₅	K ₂ O	N	P ₂ O ₅	K ₂ O
	cwt. per acre			cwt. per acre		
£ s. d.						
6 15 0	0.91	1.32	1.37 (optimal)
5 10 0	0.87	0.94	0.99 (optimal)	0.73	1.08	1.13
4 5 0	0.69	0.70	0.75	0.56	0.84	0.89
3 0 0	0.52	0.46	0.51	0.38	0.59	0.64
1 15 0	0.35	0.21	0.26	0.21	0.35	0.40

To obtain the best mixture of fertilizers for a given outlay the rule is that *the reductions below optimal should be in the ratios* N:P₂O₅:K₂O *as* 1:1.38:1.38 *or* sulphate of ammonia: superphosphate: sulphate of potash *as* 1:1.7:0.6. This rule is illustrated in Table 9 where the best mixtures for potatoes in the West Midlands and North of England are set out.

For potatoes grown with dung in West Midlands and the North of

England the best ratios of $N:P_2O_5:K_2O$ at all levels of manuring do not differ widely from 1:1:1, i.e. equal percentages of these three nutrients in terms of the statutory fertilizer analysis. For potatoes grown without dung the proportions of P_2O_5 and K_2O should be higher. It happens that the average pre-war dressing for potatoes agreed very closely in amount and proportions with the £3 per acre dressing for potatoes grown without dung, but the bulk of the potatoes were, in fact, grown with dung, and a greater proportion of nitrogen should therefore have been used.

At the present time, when synthetic nitrogenous fertilizers are being produced in this country and potash is scarce, the balance should be adjusted still further in the direction of higher nitrogen and lower potash. The war-time concentrated fertilizer containing potash provides equal percentages of N and P_2O_5 with a lower proportion of K_2O , and is therefore suitable for potatoes grown without dung. For potatoes grown with dung some of the concentrated fertilizer may well be replaced by sulphate of ammonia.

Many potato compound fertilizers contain much too low a proportion of nitrogen. One quoted in the market returns for December 1940 has the following percentage analysis: 3.5 N, 10.5 soluble P_2O_5 , 1.4 insoluble P_2O_5 , 5.0 K_2O . Such a mixture should be reinforced by sulphate of ammonia to raise the amount of nitrogen to approximate equality with that of soluble phosphoric acid.

The rates of application mentioned, of course, refer to average conditions. They may be modified for individual fields and farms in the light of former experience or soil analysis, but even these modifications should take the form of a constant amount subtracted from or added to the optimal dressings for individual crops. Poor soils or those with special deficiencies must, of course, receive extra amounts of the appropriate fertilizers.

From the national point of view the best aggregate return for a given amount of fertilizer will be obtained when all farmers with land of average fertility are working to about the same rate below the optimal dressings. If some farmers give larger dressings and others little or no fertilizer, the total returns will be considerably less. Thus, for example, if two acres of potatoes received a dressing of 0.4 cwt. N per acre, the total increase in yield may be expected to be $2 \times 1.36 \times 0.9 = 2.45$ tons, whereas if 1 acre receives 0.8 cwt. N and the other is unmanured, the expected increase is $1.85 \times 0.9 = 1.66$ tons, i.e. a reduction of 32 per cent. If the dressings are 0.2 cwt. N on 2 acres or 0.4 cwt. N on 1 acre, the corresponding reduction is 20 per cent.

Actual and Optimal Consumption of Fertilizers in Great Britain

For those concerned primarily with aggregate totals for the country as a whole, the figures already given can be built up to totals by using crop acreages and by making certain assumptions about the proportion of each crop in the three main districts which normally receives dung. Table 10 shows the gross and net values of the crop increases likely to be obtained in a normal season from the optimal rates and also from

various general levels below their optima. This Table was calculated for acreages of each crop equal to those of 1937. It gives the total amounts of fertilizer elements required for these acreages and also for additional acreages of potatoes and cereals, assuming that the additional land had the same requirements as old arable land without dung in the West Midlands and North.

TABLE 10. *Crop Increases due to Optimal and Sub-optimal Fertilizer Dressings*

	Pre-war acreages. Increased value of crops Million £		Total consumption for pre-war acreages	Consumption per additional million acres	
	Gross	Net		Potatoes	Cereals
NITROGENOUS FERTILIZER (as N)					
Optimal rates	29.2	19.4	1,000 tons N		
" " less 0.2 cwt. N per acre	27.7	19.1	163	46	20
" " " 0.3 " " "	24.2	17.4	144	36	20
" " " 0.4 " " "	19.3	14.5	114	31	16
" " " 0.5 " " "	13.1	10.2	80	26	11
			47*	21	6
PHOSPHATIC FERTILIZER (as P ₂ O ₅)					
Optimal rates	8.9	5.0	1,000 tons P ₂ O ₅		
" " less 0.15 cwt. P ₂ O ₅ per acre	7.7	4.8	131	66	12
" " " 0.2 " " "	7.1	4.6	95	58	4
" " " 0.3 " " "	6.3	4.3	80*	56	..
" " " 0.45 " " "	5.1	3.8	65	51	..
			43	44	..
POTASSIC FERTILIZER (as K ₂ O)					
Optimal rates	5.4	3.1	1,000 tons K ₂ O		
" " less 0.1 cwt. K ₂ O per acre	4.7	3.0	78	68	2
" " " 0.25 " " "	4.2	2.9	59*	63	..
" " " 0.5 " " "	2.9	2.2	44	56	..
			23	44	..

* Approximately equal to pre-war consumption.

In each section of the Table one rate of application has been marked as approximating to the pre-war total consumption of fertilizers on arable land, but the returns from the pre-war use of fertilizers were undoubtedly less than those calculated from the ideal distribution of fertilizers between crops. In the absence of information from representative surveys of the way in which farmers actually used their fertilizers, it is impossible to make a reliable estimate of the returns they attained. Under war conditions, when it is of vital importance to increase efficiency of production to the utmost, such a survey would be of the greatest value.

Table 10 shows that by increasing the use of sulphate of ammonia by 1 cwt. per acre, with a proper distribution between crops, the total additional output of crops from the pre-war acreage would be worth £11,000,000 for additional fertilizer costing £4,000,000. Of the additional nitrogen, by far the greater part would be used for cereals. Most of the newly ploughed land will need nitrogenous fertilizer, at least after the first season, and the expanded acreages, together with the need for heavier dressings all round, require a vast increase in the total consumption. In view of possible temporary shortages through difficulties of distribution during the spring fertilizer season, it may be pointed out that good results are obtained from late applications of nitrogenous top-dressings to cereals, and that there is evidence that a divided dressing

—half in early spring and half quite late—is better than the whole applied at either time.

As far as phosphate is concerned, apart from a modest all-round increase for root crops, the main needs are for a better adjustment to the requirements of individual soils, including, of course, much of the newly ploughed-up grassland which is particularly deficient in phosphate. A large increase in the potato crop will necessitate a correspondingly great increase in the use of superphosphate. The figures given in Table 10 for phosphate requirements on new land are definitely underestimates, as they were derived from average potato crops mainly on old arable land.

The potentialities of increase in the use of potash would be much less than for the other two classes of fertilizer even if extra supplies were available. Pre-war consumption in the aggregate was approaching optimal, though there was, of course, great opportunity for closer adjustment to the needs of individual soils. With greatly reduced supplies all other crops must be sacrificed to ensure a sufficiency, first for potatoes, and then for other roots, such as swedes and mangolds. (Market-garden crops, which are omitted from this discussion, also stand in high need of potash.) The potato crop must receive all available supplies both directly as fertilizer and indirectly through farmyard manure and other forms of crop residues and vegetable wastes. It is important to exploit to the full the hidden reserves of soil-potash by concentrating potatoes on land likely to have reasonable reserves from previous manuring, and by avoiding the disappointments and waste of effort which must result if potatoes are grown without potassic fertilizers on soils particularly deficient in potash. The estimation of soil reserves by analytical methods is important not only for the proper distribution of fertilizers but for the sound allocation of crops to secure the maximum utilization of the reserve fertility of our soils.

APPENDIX

The form of response curve adopted in this paper is

$$y = y_0 + d(1 - 10^{-kx}),$$

where y is the yield with a fertilizer dressing of x cwt. N, P_2O_5 or K_2O per acre, y_0 the yield with no fertilizer, d the limiting response, and k a value assumed to be constant for each of the three principal classes of fertilizer. The form of the curve implies that the response ($y_1 - y_0$) to a unit dressing of amount p of a fertilizer bears a constant ratio, 10^{kp} , to the additional response to a second unit. The latter response bears the same ratio to the further additional response obtained by the application of a third unit.

From various series of British experiments the following average values were obtained: $k_N = 1.1$, $k_P = 0.8$, $k_K = 0.8$, $k_{\text{dung}} = 0.04$ (for sugar-beet tops k_N was taken as 0.4). A later analysis of a much larger number of experiments in Sweden and Denmark confirmed the general accuracy of these values, though it suggested that some further improvement would be obtained by giving k_N a slightly higher value for cereals and a slightly lower one for roots. The values for k_P and k_K are in reasonable agreement with the corresponding constants used by Mitscherlich, but his value for nitrogen is quite inappropriate for field experiments.

The relative responses to different dressings are shown in Table 11, the response

to the standard dressing being taken as unity. In order to convert the response to any given dressing to standard it is only necessary to divide by the value of the relative response corresponding to that dressing.

TABLE 11. *Standard Response Curves for N, P, and K*

N ($k_N = 1.1$)		P and K (k_P and $k_K = 0.8$)	
Cwt. N per acre	Relative response	Cwt. P_2O_5 or K_2O per acre	Relative response
0.1	0.48	0.2	0.51
0.2	0.85	0.4	0.87
0.25	1.00	0.5	1.00
0.3	1.14	0.6	1.11
0.4	1.36	0.8	1.28
0.5	1.53	1.0	1.40
0.6	1.67	1.2	1.48
0.7	1.77	1.4	1.54
0.8	1.85	1.6	1.58
0.9	1.91	1.8	1.60
1.0	1.96	2.0	1.62

If the standard response curves are accepted as correct, the optimal dressings (in cwt. N, P_2O_5 , and K_2O per acre) are:

$$\text{For N} \quad \cdot \quad 0.12 + (\log v - \log c)/1.1,$$

$$\text{For } P_2O_5 \text{ and } K_2O \quad \cdot \quad 0.23 + (\log v - \log c)/0.8,$$

where v is the value per acre of the response to the standard dressing, and c is the cost per acre of that dressing. Table 12 shows the optimal dressings corresponding to a series of value-cost ratios, v/c .

TABLE 12. *Optimal Dressings for Various Values of v/c*

v/c	Optimal dressing in cwt. per acre of	
	N	P_2O_5 or K_2O
1	0.12	0.23
1.5	0.28	0.45
2	0.39	0.61
3	0.55	0.83
4	0.67	0.98
6	0.83	1.20
8	0.94	1.36
10	1.03	1.48

The gross values of the responses to the optimal dressings are:

$$\text{For N} \quad \cdot \quad 2.13v - 1.58c.$$

$$\text{For } P_2O_5 \text{ and } K_2O \quad \cdot \quad 1.66v - 1.09c.$$

Acknowledgements

Acknowledgements are due to D. A. Boyd, who carried out most of the computations required in the preparation of the tables; to D. J. Finney, S. G. Heintze, H. L. Penman, and E. C. R. Reeve, who assisted in the exceedingly onerous work of abstraction; and also to all members of the Rothamsted staff and others who assisted by discussion of the results and their practical implications.

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THE ROTHAMSTED EXPERIMENTS ON THE MANURING OF POTATOES

PART I. EFFECTS OF NITROGENOUS, PHOSPHATIC AND POTASSIC MANURING

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THE Rothamsted experiments on the manuring of potatoes did not begin till 1876. In the early days at Rothamsted potatoes had not been important as a farm crop, but the area had steadily expanded and by 1876 no less than 348,000 acres were grown in England and Wales.¹ Little was known, however, of the most suitable manuring for the crop: in good practice large dressings of farm-yard manure, even up to 30 tons per acre, were given, and also considerable quantities of artificials—up to 10 cwt. per acre, chiefly superphosphate or guano but sometimes also potash and nitrogen; there was, however, no great uniformity.

Two problems were studied: (i) the relative effects of nitrogenous, mineral, and farm-yard manure on the yield and composition of the potato; (ii) the influence of manuring on potato disease, this being of particular importance because *Phytophthora* was widespread, and spraying with a copper mixture had not yet come into use against it.

Gilbert discussed the results of the first twelve years of the experiments in a lecture given at Cirencester² but not in his summary, *Fifty Years Experiments*. The plan of the experiment was very simple: it consisted of ten plots in Hoosfield, and the results are set out in Table 1. The following conclusions were drawn. A complete fertilizer is essential. Nitrogen alone, whether supplied as ammonium salts or nitrate of soda, gives less increase than minerals alone, in contradistinction to mangolds where the nitrogen alone and minerals alone had given approximately the same increase; and to wheat and barley, where nitrogen alone had given a much larger increase than minerals alone. The combination of nitrogen and minerals gave a considerably better result, a full average yield, in fact, and again in contrast with other farm crops, ammonium salts was as effective as nitrate of soda.

¹ The marked change in importance of the potato crop is shown by the following acreages for England and Wales in 1876 and 1938:

	1876	1938
Area of potatoes	348,010	474,786
Total arable area	14,527,144	8,877,712
Percentage under potatoes .	2.40	5.35
Yield per acre	5.8*	7.3

* Mean 1885–94. No earlier figures published. *Bd. Agric. Returns*, 1894, p. xxvii.

² J. H. Gilbert, *Agric. Stud. Gazette*, Cirencester, 1888, 4. Rothamsted Memoirs, 6.

TABLE 1. *Yield of Potatoes, 12 years, 1876-87; and Percentage of Diseased Tubers*

<i>Artificials only</i>	<i>Yield, tons per acre</i>			<i>Percentage of diseased tubers</i>		
	<i>No minerals</i>	<i>Super.</i>	<i>Super. + K, Na, and Mg</i>	<i>No minerals</i>	<i>Super.</i>	<i>Super. + K, Na, and Mg</i>
No nitrogen	2.0	3.67	3.76	3	4	3.5
N as ammonium salts*	2.29	..	6.72	4	..	6
N as nitrate of soda .	2.62	..	6.65	5	..	7

<i>Farm-yard manure series</i>	<i>No manure</i>	<i>Farm-yard manure</i>	<i>Farm-yard manure + super.</i>	<i>Farm-yard manure + super. + nitrate of soda</i>
<i>1st 6 years 1876-81</i>				
Yield, tons per acre .	2.28	5.23	5.58	7.22
Percentage diseased .	4	5.5	6.7	12.5
<i>2nd 6 years 1882-7</i>	<i>No manure</i>	<i>No manure</i>	<i>Farm-yard manure only</i>	<i>Farm-yard manure only</i>
Yield, tons per acre .	1.69	3.05	6.26	4.01
Percentage diseased .	1.5	3	2.5	2

* A mixture of equal weights of sulphate of ammonia and muriate of ammonia supplying 86 lb. N per acre, as also did the nitrate of soda in both series of experiments.

The farm-yard-manure plots are best treated separately because of a change made in the manuring. Used alone farm-yard manure gave less yield than did complete artificials, as also did farm-yard manure + superphosphate; but when nitrate of soda was given in addition the yield rose to its maximum, 7.22 tons per acre. This was above the average yield of the time, and taken in conjunction with the recognized unsuitability of the Rothamsted soil for potatoes, it showed the effectiveness of this combination of fertilizers.

The design of the experiment does not allow a separation of the potash and the phosphate effects, and whilst recognizing the importance of potash for the production of starch, Gilbert considered that the experiments supported a manuring of dung, phosphate, and nitrogen. The discontinuance of nitrate caused a sharp reduction in yield: the small rise after omitting the superphosphate may be seasonal.

The percentage of diseased tubers varied with the luxuriance of growth: it was low (3 per cent.) on the unmanured plot and increased with the yield, rising to 12.5 per cent. on the plot receiving farm-yard manure, superphosphate, and nitrate of soda. Potash and phosphate did not reduce the liability to disease, and nitrogenous manuring increased it, though the experiment does not show whether this is the result of some modification in the composition or structure of the leaf-cell, or simply the result of the extra leaf-area.

There was a small but not very pronounced deterioration of yield. The unmanured plot which had begun with a yield of 2.6 tons for the

first four years, gave in the last five years only 1 ton per acre; but the variations in yield on the completely manured plots had not been more than could be attributed to fluctuations of season or differences in varieties. Nor was there any evidence that disease was accumulating; the percentage of affected tubers showed no tendency to rise.

The experiment was discontinued in 1901 because it was then clear that the new heavy-yielding varieties of potatoes responded well to potassic fertilizers, and it was necessary to study these in relation to the nitrogenous fertilizers.

THE MODERN EXPERIMENTS AT ROTHAMSTED AND OUTSIDE CENTRES

The modern experiments fall into two groups: continuous and shifting.

The continuous experiments.—These are made on the same land each year, but they are so designed as to eliminate the soil deterioration which complicates the classical experiments. This necessitates a rotation of crops and of treatments, and the two rotations have to be out of step. The cycle thus takes a long period to complete; in the meantime although full discussion of the results is not possible, much useful information is obtained which steadily grows in value. There are three sets of experiments in this group.

(1) *The six-course rotation* begun in 1930. The purpose is to show in their simplest form the responses to increasing amounts of each of the three main fertilizers. The data will be of particular value for the study of seasonal conditions. The order of cropping is: potatoes-rye-sugar-beet-barley-clover-wheat. There are 15 manurial treatments:

0 N, 1 N, 2 N, 3 N, 4 N each with 2 units P and K;

0 P, 1 P, 2 P, 3 P, 4 P each with 2 units N and K;

0 K, 1 K, 2 K, 3 K, 4 K each with 2 units N and P;

the single doses (1 N, 1 P, 1 K) being 0.15 cwt. N, 0.15 cwt. P_2O_5 , and 0.25 cwt. K_2O per acre. Within each of the three sets, the treatments 4, 3, 2, 1, 0 units follow each other in that order in successive years.¹ There are thus 15 plots for each crop, and 90 plots in all. The plots are not duplicated and there is no unmanured plot. No organic manure is given. The cycle is completed in 30 years, after which each plot will have completed 5 rotations by crops and 2 by treatments. The experiment is made both at Rothamsted and Woburn.

(2) *The four-course rotation* begun in 1930. The order of cropping is: potatoes-barley-rye-grass-wheat. The object is to study the effect of organic manures: farm-yard manure; straw compost; and straw ploughed in with the appropriate artificials, in comparison with artificial fertilizers. Any given plot receives always the same treatment, but the treatment is applied only once in 5 years; information is thus obtained about the effect of the fertilizer in the year of application and also in the first, second, third, and fourth years after application.² The cycle is completed in 20 years. This experiment is made at Rothamsted only.

(3) *The three-course rotation*: potatoes-barley-sugar-beet. The pur-

¹ For details see *Rothamsted Ann. Rept.*, 1932, p. 131.

² For details see *Rothamsted Ann. Rept.*, 1932, p. 127.

pose is to compare straw compost with straw ploughed in with artificials.¹

Shifting experiments.—The purpose of these is to study the manurial problems arising in actual farm practice, and they are therefore made on the ordinary farm at Rothamsted and Woburn and at a number of outside centres, mostly on the farms of good potato-growers south of the Humber and east of the Severn. Information has been obtained about the response to the three groups of fertilizers under various conditions of soil and climate, and the extent to which these responses have been affected by farm-yard manure and by the various cultivation devices. The experiments differ from the continuous series in that neither the site nor the design of the experiment has remained constant. The experiments are made under ordinary farm conditions on the field where potatoes happen to be growing; and as the method of making field experiments was improved, so the improvements were carried into the experimental design. The first experiments were on single plots, then came simple replicated experiments, and finally the modern, more complex types of factorial experiments. These are of various designs, but a frequent and useful one has 27 plots testing the effect of the three fertilizers each at three levels, 0, 1, and 2 doses.² The results are not strictly comparable with those of the older simpler experiments. The older figures show what the fertilizer did in certain conditions usually approximating to those in good practice; in particular the other nutrients were generally present in adequate amounts. The modern experiments usually include a number of treatments which would certainly not be recommended in practice but are included to measure the interdependence of fertilizer effects. When all these treatments are averaged so as to include those in which certain essential factors are omitted, the final figure is lower than it would be in a simple experiment of the old type. The results of the Rothamsted experiments as given in the following pages are only about two-thirds of what they would be if one confined attention solely to those plots receiving full quantities of the other nutrients, and which more resemble the conditions of good practice. The comparison is as follows:

	Mean response as given in tables and involving many comparisons		Response in presence of adequate basal nutrients	
	No dung	Dung	No dung	Dung
Nitrogen (0.25 cwt. N)	1.11 (8)	1.08 (10)	1.58 (8)	1.33 (10)
Phosphate (0.5 cwt. P_2O_5)	0.80 (4)	0.61 (6)	1.72 (4)	1.09 (6)
Potash (0.5 cwt. K_2O)	1.07 (5)	0.40 (8)	1.50 (5)	0.63 (8)

The figures in brackets are the numbers of experiments. Responses in tons per acre.

There are considerable objections to picking out certain plots in this way,

¹ For details see *Rothamsted Ann. Rept.*, 1933, p. 118.

² As illustration, see *Rothamsted Ann. Rept.*, 1933, p. 175.

and it is much safer to use the mean figures even though the result is lower than would be obtained in practice.

During the course of the work the fertilizer dressings have changed and where it has been desired to reduce the results to comparable form the formula of Crowther and Yates¹ has been used. These values can be distinguished from the actual experimental data since they are always given as the 'calculated increase' from 0.25 cwt. N, or from 0.5 cwt. P₂O₅, or from 0.5 cwt. K₂O per acre, as the case may be.

Effect of Nitrogenous Fertilizers on the Yield of Potatoes

It is unnecessary to give details of the first series of experiments. Table 2 shows the results of a replicated set of experiments carried out at Rothamsted for 7 successive years, 1925-31.

TABLE 2. *Effect of Sulphate of Ammonia on Yield of Potatoes*
Tons per acre, Rothamsted²

1st series. 7 years 1925-31.³

Yield without sulph. amm.	Additional yield for sulph. amm.		Calculated increase for 0.25 cwt. N
	One dose	Two doses	
7.37	1.10	1.70	1.12

2nd series. 1932-40.

Year	No farm-yard manure			Farm-yard manure				
	Yield without sulph. amm.	Additional yield for sulph. amm. cwt. per acre		Yield without sulph. amm.	Additional yield for sulph. amm. cwt. per acre		Calculated increase for 0.25 cwt. N	
		2	4		2	4	No dung	Dung
1932	9.32	1.85	3.17	10.24	2.34	3.22	1.54	1.74
1934	8.95	1.35	1.65	11.02	1.59	1.88	0.94	1.10
1935	5.56	..	2.57	7.10	..	2.06	1.39	1.11
1936	4.83	1.30	..	7.06	0.24	..	0.96	0.18
1937	4.59	1.85	2.87	6.84	1.89	3.46	1.46	1.63
1938	9.16	0.92	2.68	12.28	1.42	2.22	1.06	1.12
1939	5.93	1.99	1.86	9.70	1.46	2.14	1.24	1.12
1940	7.52	0.40†		8.83	0.72†		0.24	0.43
Mean in- crease*	7.59	1.59	2.45	10.01	1.74	2.37	1.25 1.10‡	1.34 1.04‡

* Omitting 1935, 1936, and 1940, when only single doses were given.

† 0.6 cwt. N = 3 cwt. sulphate of ammonia approximately.

‡ Mean of all results (8 expts.).

¹ E. M. Crowther and F. Yates, this Journal, 1941, 9, 77-97. This equation gives similar results to that of Mitscherlich for P₂O₅ and K₂O, but considerably different values for nitrogen.

² In all the Rothamsted experiments the potatoes are grown in rows 27 in. apart and spaced at 2 links (15.8 in.); there would thus be 14,670 plants per acre if all survived. Great care is taken about seed and cultivations, and the loss of plant is small.

³ The figures are the means of all potash and phosphate treatments. Dung was given in each year except 1925 and 1931.

Six-course rotation: no dung: average 11 years 1930-40.

	Yield without sulph. amm.	Additional yield for N as sulph. amm.			
		0.15 cwt. N	0.30 cwt. N	0.45 cwt. N	0.60 cwt. N
Rothamsted . . .	5.79	0.75	0.83	0.86	1.27
Woburn . . .	6.31	0.86	1.66	2.65	2.67

(0.15 cwt. N = 0.75 cwt. sulphate of ammonia)

Increasing the dressing of sulphate of ammonia increases the yield, though not as a rule proportionately; in most years the increase given by 4 cwt. of the fertilizer would be profitable.

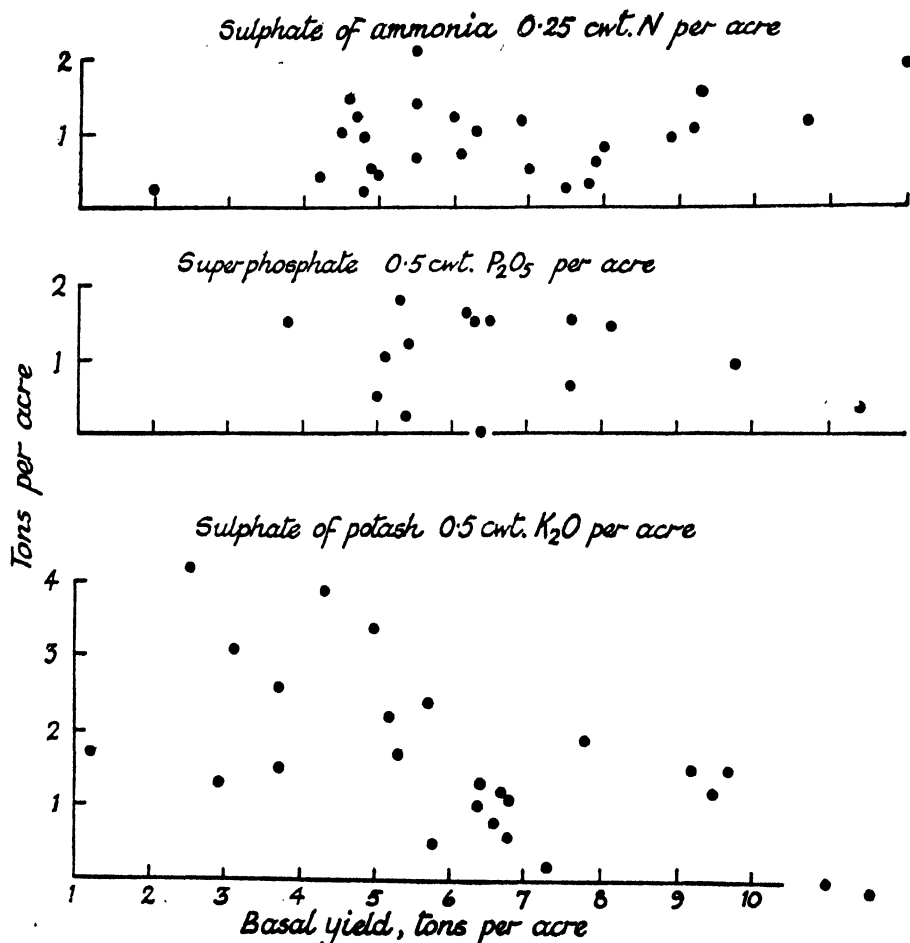


FIG. 1. Responses to unit dressings plotted against basal yields (Rothamsted). No dung.

The increments vary from year to year, but less than those for the standard dressings of phosphate and of potash (Fig. 1). They show no relation to the initial yield, and they are not affected by farm-yard manure.

Further they were substantially the same in the early years when the land was known to be in poor condition, as in the later years when it was much better; the average calculated increases per 0.25 cwt. nitrogen were:

1925-31	.	.	.	1.12 tons potatoes
1932-40	.	.	.	1.08 " "

Even as between the original Lawes and Gilbert experiments and our latest set the difference is not great in spite of the wide difference in varieties: their average increment for 4 cwt. sulphate of ammonia was 3.2 tons whilst ours was 2.45 tons potatoes.

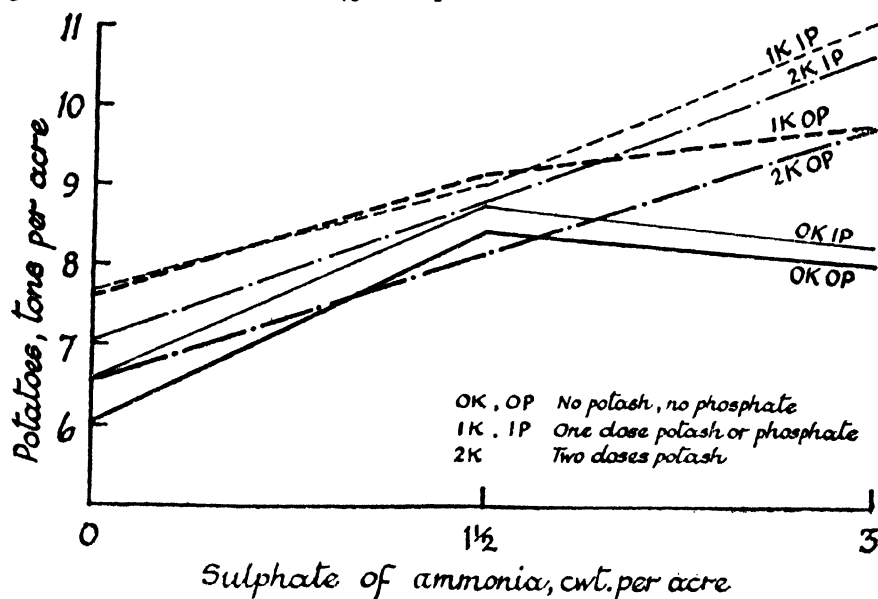


FIG. 2. Effect of fertilizers on yield of potatoes.

TABLE 3. *Effect of Phosphatic and Potassic Fertilizers on the Action of Nitrogenous Fertilizers. (Rothamsted)*

		No super. K ₂ O cwt. per acre			Super. (0.5 cwt. P ₂ O ₅ per acre) K ₂ O cwt. per acre		
		0	0.5	1.0	0	0.5	1.0
1928	..	0	0.5	1.0	0	0.5	1.0
Nitrogen	0	6.09	7.62	6.58	6.60	7.67	7.06
cwt.	0.3	8.42	9.15	8.13	8.75	9.03	8.79
per acre	0.6	8.00	9.76	9.74	8.26	11.05	10.63
1931	..	0	0.4	0.8	0	0.4	0.8
Nitrogen	0	10.11	10.79	11.33	11.02	10.69	10.27
cwt.	0.2	11.35	11.23	11.65	11.78	11.65	12.10
per acre	0.4	12.26	11.94	11.97	13.11	12.08	12.87

Action of potassic and phosphatic fertilizers on the effectiveness of nitrogenous fertilizers.—The figures in Table 2 are the means for all plots

receiving the specified nitrogenous dressing, but in poor soil conditions the effectiveness of the nitrogen is enhanced by phosphate and potash. In more fertile conditions phosphate still enhances the effect, though potash does not. The two cases are illustrated in Table 3. The 1928 experiment was on poor soil and the basal yield without fertilizer was only 6 tons per acre: this was raised to 9.7 tons by the addition of sulphate of potash and to 10.6 tons by the further addition of superphosphate, but only at the higher, not at the lower, levels of nitrogenous manuring. This important point is illustrated in Fig. 2: the crop increases for the first dose of nitrogen are much the same for all treatments, but those for the second show a wider spread. The 1931 experiment, on the other hand, was made in much more productive conditions, and although the increment given by the nitrogen is substantially the same as in the earlier experiment, there is no enhancement of the effect by potash though there is by phosphate.

The general effects at Rothamsted are shown in Fig. 3.

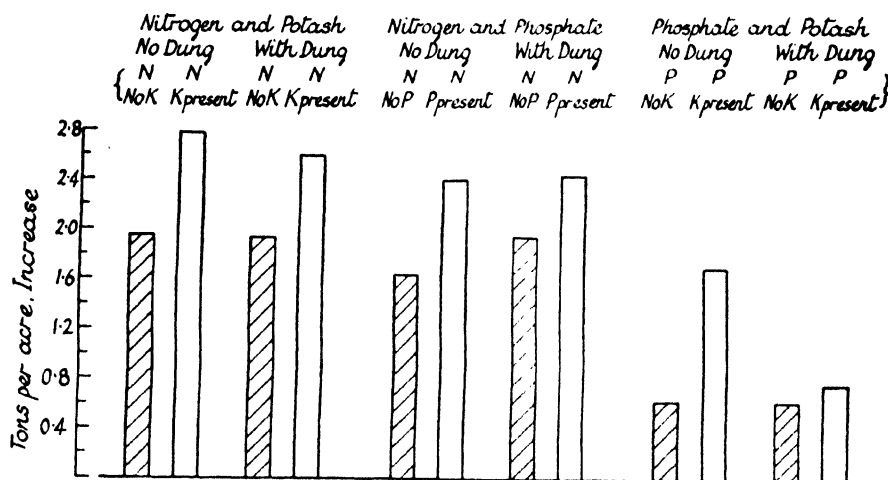


FIG. 3. Action of one fertilizer on the effectiveness of another (Rothamsted).

The average enhancement of the nitrogen effect by potash at Rothamsted has been, in tons per acre:

	Yield No N, no K	Increase given by			Enhancement† of nitrogen effect by potash
		N, no K	K, no N	N+K	
No dung*	7.26	1.94	1.04	3.82	0.84
Dung†	8.02	1.93	0.09	2.69	0.67

* 1925-39, 0.7 cwt. N and 1.5 cwt. K_2O per acre; 6 experiments.

† 1926-39, 0.6 cwt. N and 1.5 cwt. K_2O per acre; 9 experiments.

‡ The enhancement is $(N+K) - N - K = 3.82 - 1.94 - 1.04 = 0.84$.

The enhancement by phosphate at Rothamsted has been, in tons per acre:

	Yield No N, no P	Increase given by			Enhancement of N effect by P
		N, no P	P, no N	N+P	
No dung*	6.68	1.63	0.45	2.85	0.77
With dung†	7.88	1.94	0.51	2.95	0.50

* 5 experiments: 0.7 cwt. N and 0.7 cwt. P_2O_5 per acre.

† 6 experiments: 0.7 cwt. N and 0.6 cwt. P_2O_5 per acre.

A similar enhancement both by potash and by phosphate is seen at the outside centres; it is more pronounced for potash on heavy than on light soils except in the fens (Table 4).

TABLE 4. *Summary showing Enhancement* of Nitrogen Effect by Potassic and Phosphatic Fertilizers at Outside Centres. Tons per acre*

Potassic Fertilizers						
Mineral soils					Fen soils	
	Light	Medium	Silt	Heavy	Light	Heavy
All centres:						
No dung . . .	0.42 (9)	0.10 (3)	-0.12 (4)	1.06 (3)	0.44 (13)	0.04 (9)
Dung . . .	-0.38 (2)	0.88 (1)	-0.18 (3)	0.72 (1)	0.02 (6)	0.88 (2)
Experiments showing definite responses to potash:						
No dung . . .	-0.08 (4)	0.68 (2)	..	1.06 (3)	0.52 (12)	0.08 (1)
Dung	0.88 (1)	Nil (1)	0.72 (1)	0.34 (3)	0.20 (1)
All centres:	Phosphatic Fertilizers					
No dung . . .	-0.04 (11)	0.54 (6)	0.96 (1)	0.86 (3)	0.36 (9)	0.78 (8)
Dung . . .	0.78 (1)	..	0.42 (3)	..	0.10 (4)	2.00 (2)
Experiments showing definite responses to phosphate:						
No dung . . .	0.44 (4)	0.86 (2)	0.96 (1)	1.20 (2)	0.92 (5)	0.78 (8)
Dung . . .	0.78 (1)	..	0.88 (1)	..	0.58 (2)	2.00 (2)

* In the Rothamsted Reports the enhancement is halved and called 'interaction' (see *Roth. Ann. Rept.*, 1936, pp. 170-3); this mode of presentation has advantages from the statistical point of view, but for our present purpose the actual experimental figure is preferable.

The enhancing action of phosphatic fertilizers on the effectiveness of nitrogen is usually rather greater than that of potash except on the heavy soils, where, as at Rothamsted, the effect is reversed.

Action of farm-yard manure on the effectiveness of nitrogenous fertilizers.—Farm-yard manure has practically no action on the effectiveness of sulphate of ammonia at Rothamsted (Table 2). The increments are:

	Without dung	With dung
For 2 cwt. sulphate of ammonia per acre . . .	1.59	1.74
" 4 " " " " " " . . .	2.45	2.37

The same result is obtained also at the outside centres, as shown in Table 6: only on the light soils did any marked difference appear. A typical experiment at the Seale Hayne College in 1927 gave:

No farm-yard manure				Farm-yard manure			
No sulph. amm.	Additional yield for sulph. amm. cwt. per acre			No sulph. amm.	Additional yield for sulph. amm. cwt. per acre		
	1	2	3		1	2	3
3.70	1.50	2.52	2.60	5.32	1.36	2.27	2.82

Calculated increase for 0.25 cwt. N 1.73 1.66

At other centres where there was a direct comparison the results were:

	Without dung		With dung	
	Yield without sulph. amm.	Additional yield for sulph. amm.	Yield without sulph. amm.	Additional yield for sulph. amm.
6 Northumberland and Durham centres*	5.77	0.61	8.17	0.49
Light Fen, Wimblington†:				
3 years	6.14	0.31	8.68	0.99
5 centres‡ 1940 . .	9.04	0.20	9.72	0.33

* 2 cwt. each sulphate of ammonia and of potash, 4 cwt. superphosphate; 12 tons dung per acre where given.

† Rothamsted Reports, 1934, p. 229; 1935, p. 258; 1936, p. 262. 0.5 cwt. N.

‡ 0.6 cwt. N.

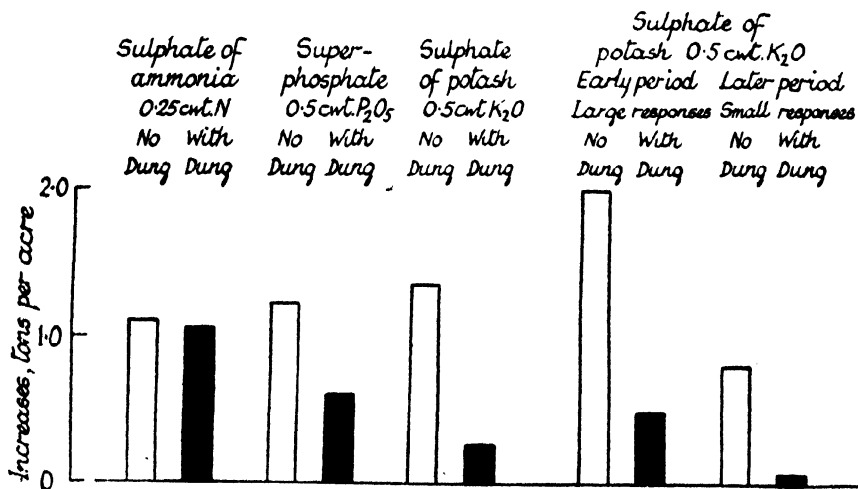


FIG. 4. Effect of dung on the action of artificial fertilizers (Rothamsted).
Increases, tons per acre.

We are unable to account for this remarkable result. The effect of a given quantity of sulphate of ammonia is diminished when more sulphate of ammonia is added, but not when dung is added, yet both increase the

supply of nitrogen. Part of the effect may be due to the potash in the farm-yard manure, which as shown in Fig. 3 enhances the effect of the nitrogen, but something more seems to be involved. Whatever the action, it is peculiar to nitrogen, for the farm-yard manure depressed the effect of phosphate and of potash just as additional fertilizer would do (Fig. 4).

Influence of soil type on the effectiveness of nitrogenous fertilizers.—The simplest comparison showing the effect of soil type is that of Rothamsted and Woburn in the 6-course rotation experiment (p. 197), and the results are in tons per acre:

	Basal yield No nitrogen	Mean increment for 0.25 cwt. N per acre	Annual variation (S.E. per year)
Heavy soil (Rothamsted) .	5.79	0.64 ± 0.10	± 0.32
Light soil (Woburn) .	6.1	1.73 ± 0.30	± 1.00

The similarity in basal yields is striking, but it is confirmed in the outside experiments, where if the rich silt and the heavy fen are excluded the effect of soil type on yield is seen to be small (Table 5).

The increment per unit dressing of nitrogen is larger at Woburn than at Rothamsted. In general at other centres the light soils are not more but usually less responsive than the medium and heavy ones.

For annual variation we have figures only for Rothamsted and Woburn,

TABLE 5. *Effect of 0.25 cwt. Nitrogen as Sulphate of Ammonia on Soils of Different Types (1921–40). Yield of potatoes, tons per acre*

	No dung			Dung		
	No. of experi- ments	Yield No N	Calcu- lated increase for 0.25 cwt. N	No. of experi- ments	Yield No N	Calcu- lated increase for 0.25 cwt. N
Light . .	34	7.71	0.74	15	8.48	0.32
Medium . .	41	6.62	1.20	9	8.02	0.95
Rich silt* . .	2	12.08	0.52	4	11.02	1.20
Heavy . .	20	7.11	0.76	9	7.60	0.76
Rothamsted . .	15	7.07	1.11	14	8.08	1.16
Light fens . .	13	7.19	0.55	7	9.17	0.71
Heavy fens . .	8	9.49	1.61	3	8.55	1.30
Totals and means	133	8.18	0.93	61	8.70	0.91

* This soil has been exceptionally heavily manured for many years: potatoes have been taken every third year.

and the light soil shows greater variability in response to nitrogen than the heavy one. This happened also in the continuous experiments on wheat and barley.¹

The response to nitrogen has but little relation to the basal yield. It

¹ *Fifty Years of Woburn Experiments*. E. J. Russell and J. A. Voelcker. Rothamsted Monographs on Agricultural Science, Longmans & Co.

varies from year to year, but whatever the causes, they do not appear to be the same as those governing the variations of the basal yield (Fig. 1).

The effect of farm-yard manure cannot properly be seen from Table 5 because the numbers of experiments are not the same in the two sections: it is dealt with later. The heavy fen soils and the silt did not respond to dung, though the others did. On none of the soils where the comparison was valid, however, did the farm-yard manure appreciably affect the response to sulphate of ammonia.

The small effect of soil type on basal yield, apart from the heavy fen and the rich silt, shows that no one soil type is markedly better for the growth of potatoes than any other, though the heavy fen soil is actually the most responsive. One important factor, however, does not come into account here. The considerable amount of cultivation necessary for the growth of potatoes which is given as a matter of course in the experiments becomes of primary importance in practice, and hence potato-growing in peace-time is largely restricted to soils easy to cultivate.

In some of the experiments the sulphate of ammonia was given at two different rates. The results were very similar to those at Rothamsted: the first dose gave a substantial increase and the double dose gave a further good, but not a doubled, increase (Table 6), and as in the experiments set out above, the heavy fen soils were the most responsive to both dressings and the light soils were least responsive (Fig. 5).

Other Nitrogenous Compounds

Calcium cyanamide was tested at Rothamsted for several years, but it gave only about 60 per cent. of the response from sulphate of ammonia supplying equal amounts of nitrogen (0.6 cwt.) per acre. The average result of all experiments¹ was:

Number of expts.	Yield, tons per acre			Response to nitrogen in calcium cyanamide when that to sulph. amm. = 100
	No nitrogen	Increase for		
		Sulph. amm.	Cyanamide	
6	7.36	1.66	0.97	59

There is therefore no reason why calcium cyanamide should be used for potatoes.

Ammonium bicarbonate.—At one period it appeared probable that at many of the smaller gas-works the bicarbonate could be more conveniently prepared than the sulphate of ammonia. Unfortunately it decomposes readily giving off ammonia and so is liable to loss: it therefore does not give as good returns as the sulphate:

Number of expts.	Yield, tons per acre			Response to nitrogen in bicarbonate when that to sulphate = 100
	Increase for 0.6 cwt. N as			
	No nitrogen	Sulphate	Bicarbonate	
9	8.46	1.06	0.61	57

¹ Rothamsted Ann. Repts., 1927, p. 155, and subsequent issues till 1931, p. 192.

Muriate of ammonia.—This salt could have been made on the large scale as an alternative to the sulphate, and experiments with barley suggested that it possessed some superiority. On potatoes, however, it was

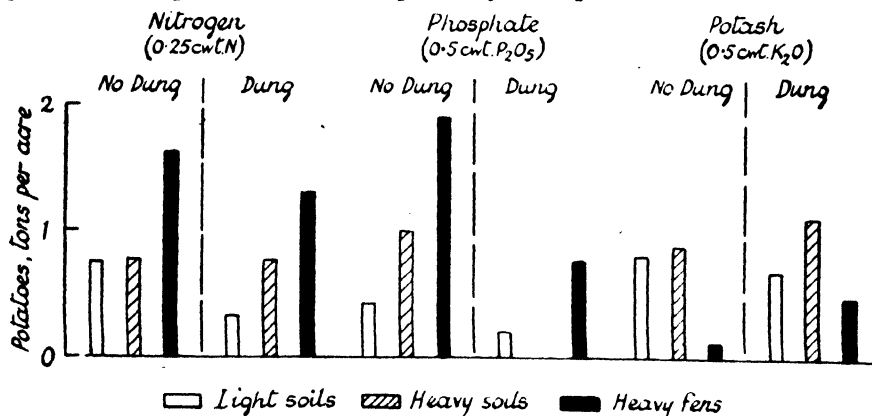


FIG. 5. Fertilizer responses on different soil types. Increases for the standard dressings.

TABLE 6. *Effect of Increasing Dressings of Sulphate of Ammonia on Different Soils*

Soil type	No. of experiments	Yield No N tons per acre	Increase for sulph. amm., tons per acre	
			One dose (1½–2 cwt. per acre)	Two doses (3–4 cwt. per acre)
Light and medium soils				
No dung . . .	11	9.02	0.88	1.41
With dung . . .	7	9.22	0.66	0.76
Rothamsted				
No dung . . .	7	8.00	1.60	2.49
With dung . . .	8	8.13	1.60	2.37
Light fen				
No dung . . .	6	8.18	1.15	1.64
Heavy fen				
No dung . . .	5	8.96	2.21	3.21

less successful because its chlorine¹ is harmful: with a mean dressing of 0.4 cwt. N per acre the results were:

Yield, tons per acre

	No. of experiments	No nitrogen	Increase for		Response to nitrogen in muriate when that in sulphate = 100
			Sulphate	Muriate	
Dung . . .	23	8.67	0.96	0.79	82
No dung . . .	17	5.76	1.54	1.31	85

¹ It contains 66 per cent. Cl.

The mean depression per cwt. of Cl is about the same as for muriate of potash. As in that case also the depression is greater in the drier years than in the wetter ones, but there was no marked drought in any of the years of experiment, and we cannot say whether any reversal of the effect would in those conditions be obtained as seems to happen with muriate of potash (v. Part II). In the wet years the reduction of the depression apparently went further than for the muriate of potash, giving a small increase in yield, but as the experiments were all of the old type we cannot be sure that this particular effect is real, though there seems no doubt about the increased harmfulness in the drier seasons:

Yield with sulphate, tons	8.02	9.75	10.69	9.54	9.44
Muriate*: increase (+) or decrease (—)	+0.61	+0.23	—0.13	—0.19	—0.30
Rainfall, April–July, inches†	14.3	10.74	10.71	8.73	7.65
Year and no. of centres	1924 (3)	1922 (6)	1926 (3)	1925 (3)	1923 (3)

* Equal quantities of nitrogen used, except in 1925 and 1926 when equal weights of fertilizer were given.

† At Rothamsted.

Effect of Superphosphate on the Yield of Potatoes

The Rothamsted experiments with superphosphate are summarized in Table 7. The basal yields have varied from 5.1 to 12.9 tons per acre and the increases, calculated to 0.5 cwt. P_2O_5 per acre, from 0.20 to 1.47 tons per acre; the variability in effect is thus greater than in the case of nitrogen. There is a small but not significant tendency for the increases in absence of dung to be higher in years of low basal yield (Fig. 1).

The increments given by superphosphate are increased by the addition of nitrogenous and of potassic fertilizers; this is dealt with on pp. 201 and 212, and it is well shown in the experiments of 1938, the results of which were:

Sulphate of ammonia (cwt. N)	No Dung				Dung			
	No super.		Increase due to 5 cwt. super.*		No super.		Increase due to 5 cwt. super.	
	Sulph. pot. (cwt. K_2O)		Sulph. pot. (cwt. K_2O)		Sulph. pot. (cwt. K_2O)		Sulph. pot. (cwt. K_2O)	
	0	1.6	0	1.6	0	1.6	0	1.6
0	8.18	9.27	+0.20	+1.56	11.65	11.73	+1.53	+0.82
0.4	8.15	9.80	+1.09	+3.34	13.78	12.77	—0.05	+1.77
0.8	11.87	11.42	—2.51	+3.28	13.99	13.50	+0.58	+2.44

* 0.8 cwt. P_2O_5

Action of farm-yard manure on the effectiveness of superphosphate.—In striking contrast with the results on p. 203 farm-yard manure depresses the effectiveness of superphosphate notwithstanding that much of its action is determined by its nitrogen and its potash. The depression appears on all types of soil (Table 9) and its actual amount is shown in Table 8,

which summarizes the experiments designed to measure it. At Rothamsted 15 tons of dung per acre supplying 0.9 cwt. P_2O_5 halved the effect of 0.8 cwt. P_2O_5 as superphosphate; at Tunstall also the effect was halved, but the action was more variable at the other centres (Fig. 4).

TABLE 7. *Increases in Yield given by Superphosphate (Rothamsted). Calculated Values for 0.5 cwt. P_2O_5 per acre; tons per acre*

	1928	1929	1930	1931	1933	1937	1938	1939	Mean
<i>No dung</i>									
Yield without phosphate	11.40	5.01	5.40	9.78	6.29	7.58
Increase for 0.5 cwt. P_2O_5	0.33	0.49	1.19	0.90	1.47	0.88
<i>Dung</i>									
Yield without phosphate	8.17	5.12	8.57	8.20	12.90	10.77	8.96
Increase for 0.5 cwt. P_2O_5	0.49	0.58	0.80	0.66	0.93	0.20	0.61

<i>Woburn*</i>	1927	1928	1929	1930	Mean
Yield without phosphate	4.06	12.25	5.05	11.11	8.12
Increase for 0.5 cwt. P_2O_5	Nil	1.36	-0.02	-0.22	0.28

* Dung was given in each year except 1929.

Six-course rotation: no dung: average 11 years 1930-40, tons per acre

	Yield without phosphate	Additional yield for P_2O_5 cwt. per acre			
		0.15	0.30	0.45	0.60
Rothamsted	6.47	-0.28	0.45	0.67	0.69
Woburn	7.31	0.41	0.88	0.83	0.57

(0.15 cwt. P_2O_5 = 1 cwt. superphosphate)

Action of soil type on the effectiveness of superphosphate.—The response to phosphate is greater on the heavy soil at Rothamsted than on the light soil at Woburn, and this appears to hold generally (Table 9). On the other hand, the annual variation is about the same at the two centres, but whereas at Woburn it shows no relation to the basal yield there is an indication that at Rothamsted the phosphate has been more effective in the less productive seasons (Fig. 1).

	Basal yield No phosphate	Mean increment for 0.5 cwt. P_2O_5 per acre	Annual variation (S.E. per year)
Heavy soil (Rothamsted)	6.47	0.84 ± 0.33	± 1.10
Light soil (Woburn)	7.31	0.57 ± 0.33	± 1.08

The heavy fen soils are the most responsive: on these an increase of nearly 2 tons per acre was obtained for a dressing of 0.5 cwt. P_2O_5 . Heavy

mineral soils gave about half this increase, but light mineral soils gave less (Table 9).

This varying response on different soil types does not depend on the general fertility of the land, as shown by the mean yield, for some of the highest responses are obtained on soils so rich that even without added phosphate they still give high yields per acre: this is in sharp contrast to potash, which has but little effect in more productive conditions.

TABLE 8. *Effect of Superphosphate in Presence and Absence of Dung. Potatoes, tons per acre*

Year	Centre	No dung		With dung		Manure per acre	
		Yield without phosphate	Increase given by phosphate	Yield without phosphate	Increase given by phosphate	P ₂ O ₅ cwt.	Dung tons
1937	Rothamsted	5.40	1.52	8.20	0.85	0.8	15
1938	Rothamsted	9.78	1.26	12.90	1.19	0.8	15
1939	Rothamsted	6.29	1.88	10.77	0.26	0.8	15
	Mean*	7.16	1.55	10.62	0.77	0.8	15
1935	Wimblington	5.66	0.49	8.16	0.45	1.0	8
1936	Wimblington	7.64	0.03	9.24	-0.80	1.0	8
	Mean	6.65	0.26	8.70	-0.18	1.0	8
1937	Tunstall	5.79	0.64	6.31	0.36	1.0	10
1940	Cudworth	6.14	0.79	7.66	0.15	0.64	10

* For standard dressing of 0.5 cwt. P₂O₅ the increases are: no dung 1.19, dung 0.60.

TABLE 9. *Response to Superphosphate on Soils of Different Types*

Increase yield of potatoes (tons per acre) given by 0.5 cwt. P₂O₅ per acre

Soil type	No dung			With dung		
	No. of experiments	Yield without phosphate	Calculated increase given by phosphate	No. of experiments	Yield without phosphate	Calculated increase given by phosphate
Light . .	18	7.85	0.42	7	8.35	0.20
Medium . .	9	4.38	0.79	3	13.21	0.96
Rich silt . .	2	12.36	0.82	8	13.14	0.45
Heavy . .	5	6.22	1.03
Rothamsted . .	5	7.22	0.88	6	8.96	0.61
Light fen . .	10	6.76	0.78	6	9.05	0.15
Heavy fen . .	9	9.51	1.90	2	8.05	0.76
Totals and means	58	7.76	0.95	32	10.13	0.52

A double dressing of superphosphate gave a larger yield than a single one though not so large proportionately as was given by nitrogen. The heavy fen soil gave the largest increases both for the single and the

double dressings and at Owmbly (Lincolnshire Cliff) there was no increase for either. On the light fen the effect of superphosphate was much reduced by dung, but nevertheless the double dressing gave a larger return than the single one (Table 10).

TABLE 10. *Effect of Single and Double Dressings of Superphosphate on Different Soils*

	Yield No P	Increase for super.	
		4-5 cwt.	8-10 cwt.
Wisbech silt with and without dung (9 years)	12.99	0.64	0.74
Owmbly, Lincs., cliff land with and without dung (4 years)	8.50	-0.02	-0.12
Light fenland			
No dung (6)	7.48	1.28	1.61
With dung (4)	9.23	0.29	0.51
Heavy fenland			
No dung (8)	9.23	2.40	3.40

(The numbers in brackets are the numbers of experiments)

Effect of Gafsa phosphate.—Gafsa phosphate is compared with superphosphate in the four-course rotation experiment (p. 197), but it always comes out inferior.

	1st application	2nd	3rd	4th
Superphosphate	5.57	3.96	4.16	3.88
Gafsa phosphate	3.12	2.74	3.48	3.40

Dressings of 1.8 cwt. sulphate of ammonia and 1.2 cwt. muriate of potash per acre were given also, but no organic manure.

Effect of Potassic Fertilizers

THE marked effect of potassic fertilizers on potatoes was demonstrated as early as 1870 by Augustus Voelcker in a series of farm experiments made in different parts of England,¹ but was unfortunately not tested in the early Rothamsted experiments.

One of the most striking results of the modern experiments both at Rothamsted and the outside centres has been the variability of the effect of potassic fertilizers according to the conditions. The increments in the Rothamsted experiments of 1925-31 are set out in Table 11; they fall more rapidly with the second and third doses than in the case of nitrogen. Taking the experiments as a whole the response to potash is more variable than that to phosphate or nitrogen (Fig. 1, p. 200), but a good deal of this is due to soil conditions as shown later. In the six-course rotation where these are eliminated the annual variation is no greater than for phosphate, but the responses are related to the basal yield, being higher in the bad seasons than the good ones. The experiment has not

¹ *J. Roy. Agric. Sci. Eng.*, 1870, 6, 392.

TABLE 11. *Effect of Increasing Doses of Sulphate of Potash on Yield of Potatoes (Rothamsted). Tons per acre*

Year*	Yield without potash	Additional yield for sulph. pot.			K ₂ O in smallest dose cwt.
		One dose	Two doses	Three doses	
1925	5.73	..	3.14	4.08	1.0
1926	8.62	1.00	1.16	1.28	0.5
1927	6.92	..	0.46	0.43	1.0
1928	7.69	1.36	0.80	..	0.5
1929	5.21	0.14	0.31	..	0.5
1930	8.40	0.94	1.15	..	0.4
1931	11.60	-0.26	0.43	..	0.4

Six-course rotation: no dung: average 11 years 1930-40.

	Yield without potash	Additional yield for K ₂ O, cwt. per acre			
		0.25	0.50	0.75	1.00
Rothamsted .	4.99	1.58	1.99	2.15	2.63
Woburn .	7.68	0.0	0.78	0.63	0.73

(0.50 cwt. K₂O = 1 cwt. KCl)

* Rothamsted Ann. Repts., 1925, p. 139; 1926, p. 140; 1927, p. 141; 1928, p. 143; 1929, p. 99; 1930, p. 145; 1931, p. 154.

continued long enough to show how the responses are affected by the various weather conditions. In the shifting experiments, which are more numerous, the effects of soil conditions override other factors and no relation can be traced with hours of sunshine or of rainfall.¹

Action of nitrogenous and phosphatic fertilizers on the effectiveness of potassic fertilizers.—The enhancement of the potash effect by sulphate of ammonia is shown on p. 202; superphosphate also has a similar but greater effect in absence of dung, especially on the light fen, and even on the silts and light soils where sulphate of ammonia caused no enhancement; in presence of dung, however, superphosphate is without effect on the action of the potash (Table 12).

TABLE 12. *Effect of Superphosphate on the Effectiveness of Potassic Fertilizers. Tons per acre*

	Rothamsted	Mineral soils			Fen soils		
		Heavy	Medium	Light	Silt	Heavy	Light
<i>All centres:</i>							
No dung	1.07 (5)	0.06 (2)	-0.24 (4)	0.12 (10)	0.80 (2)	-0.66 (7)	1.04 (10)
Dung .	0.14 (5)	-0.74 (1)	-0.10 (3)	-0.48 (2)	0.48 (4)
<i>Experiments showing definite responses to potash:</i>							
No dung	1.50 (4)	2.42 (1)	0.96 (1)	0.48 (6)	1.14 (1)	0.30 (2)	1.06 (7)
Dung .	-0.14 (2)	-0.42 (1)	-1.12 (1)	0.24 (1)

¹ In the earlier years of the experiments there seemed to be indications of increased effectiveness of potassic fertilizers in sunless seasons, but in the fuller series this does not appear, though whether this is the result of the change described on p. 214 cannot at present be determined.

The details of the Rothamsted results are:

	Yield		Increase given by			Enhancement of P-effect by K
	No P	No K	P, no K	K, no P	P+K	
No dung*	7.66		0.62	0.60	2.29	1.07
With dung†	9.22		0.62	0.03	0.79	0.14

* 5 experiments. 0.8 cwt. P_2O_5 and 1.3 cwt. K_2O per acre.

† 4 experiments. 0.7 cwt. P_2O_5 and 1.4 cwt. K_2O per acre.

Depressing action of farm-yard manure.—In sharp contrast with sulphate of ammonia, the effectiveness of potassic fertilizer, like that of superphosphate, is greatly reduced by farm-yard manure. All experiments show this: the average result of all the modern replicated experiments at Rothamsted and outside centres, where dung had been one of the experimental treatments, was:

	No dung	Dung	Increase for dung
No potash	8.08	10.32	2.24
K_2O , 0.5 cwt. per acre	9.16	10.53	1.37
Increase for potash	1.08	0.21	
Depression of potash effect by dung	0.87		Depression of dung-effect by potash = 0.87

The nitrogen and phosphate present in the dung might have been expected to enhance the effect of the potassic fertilizer, but in spite of this the total action of the dung is very depressing. A probable explanation is the large amount of K_2O present in the dung, a 15-ton dressing supplying some 225 lb. But this applies also to the nitrogen, and yet in that case there is no depression.

The depression is of course mutual, and it is equally true to say that potassic fertilizers depress the action of dung as to say that dung depresses the action of potash.

Effect of soil conditions on the action of potassic fertilizers.—The soil conditions profoundly affect the action of potassic fertilizers on potatoes: even on the same farm and in the same season different fields give different responses. At Rothamsted the calculated responses to 0.5 cwt. K_2O were, in tons per acre:

	1939			1940		
	Gt. Knott	Long Hoos II	Long Hoos	West Barn	Gt. Harpenden	Long Hoos
No potash	6.85	6.40	5.25	7.84	6.76	5.30
Increase for potash	0.63	1.05	2.20	1.91	1.10	1.71

It commonly happens that a soil in poor condition responds better to potash than an otherwise similar soil in good condition. The first of the Rothamsted experiments were made on poor fields only recently

taken over; they gave poor basal yields but good responses. As the land was improved, however, the basal yields went up but the responses went down:

Period*	No. of seasons	No dung		Dung	
		Yield without potash	Calculated increase for 0.5 cwt. K ₂ O	Yield without potash	Calculated increase for 0.5 cwt. K ₂ O
1921-4 .	4	5.01	2.00	8.10	0.49
1932-40 .	5	7.83	0.81	10.73	0.07
Mean .	9	6.58	1.34	9.56	0.26

* The 1921-4 experiments were of the old replicated type; the later ones in 1931-2 and 1937-40 were of modern factorial type.

At the outside centres very productive soils capable of yielding 10 tons or more of potatoes without additional potash showed little benefit from the use of potassic fertilizer. At three of the centres potatoes heavily manured with dung and artificials had long formed part of the cropping scheme; at all of them the effect of potassic fertilizer has been small.

Soil	Location	No. of experiments	Mean yield tons per acre	Mean increase for 0.5 cwt. K ₂ O
Silts . . .	Wisbech area	5	11.18	0.18
Clay fen . . .	Littleport	10	10.18	0.19
Fertile light soil	Sutton Bonnington	6	9.2	0.16

Effect of soil type.—The marked variation in effect of potassic fertilizers with the soil conditions makes it difficult to generalize about the influence of soil type. In the six-course rotation the response to potash on the light soil at Woburn is much less than on the heavy soil at Rothamsted: it is also less variable and quite differently related to the initial yield:

	Basal yield No potash	Mean increment per 0.5 cwt. K ₂ O per acre	Annual variation (S.E. per year)
Heavy soil (Rothamsted) .	4.99	1.70 ± 0.32	± 1.06
Light soil (Woburn) .	7.68	0.61 ± 0.18	± 0.61

Reference to Table 13 shows that the Rothamsted response is unusually high while that at Woburn is not markedly different from what is obtained on other light mineral soils. But there is no evidence at these particular centres for the common statement that light soils respond much better than heavy ones to potash excepting only on the light and heavy fen. The latter is the least responsive of all soils examined; the other relatively unresponsive is the silt: both are obviously in high condition as shown by the high initial yield, and in these circumstances as we have already seen potash does not act well.

TABLE 13. *Mean Effect of 0.5 cwt. K₂O on Yield of Potatoes on Soils of Different Types in Presence and Absence of Dung*

	No dung			Dung		
	No. of experiments	Yield without potash	Calculated increase for 0.5 cwt. K ₂ O	No. of experiments	Yield without potash	Calculated increase for 0.5 cwt. K ₂ O
Mineral soils:						
Light	22	7.25	0.80	16	9.11	0.68
Medium	15	7.81	0.98	8	8.88	0.90
Rich silt	1	12.87	0.34	3	12.07	0.26
Heavy	8	6.81	0.89	11	7.33	1.11
Rothamsted	15	5.92	1.76	15	9.33	0.45
Fen soils:						
Light	15	6.67	1.73	7	9.08	0.79
Heavy	8	10.63	0.12	2	8.35	0.48
Totals and means	84	8.28	0.95	62	9.18	0.67
Weighted mean	1.11	0.71

Cross comparisons between effects in presence and absence of dung cannot be made except at Rothamsted, as different centres and years are involved.

As at Rothamsted, the second dose of potassic fertilizer is much less effective than the first and only on the light fen soils and at Rothamsted without dung has it been economically advantageous. The heavy fen and the silt have been very unresponsive (Table 14).

 TABLE 14. *Effect of Increasing Dressings of Sulphate or Muriate of Potash*

Soil type	No. of experiments	Yield No K	Increase for sulphate or muriate of potash			
			1-1½	1½-2	2-3	3-4 cwt.
Light and medium:						
No dung	12	9.12	+0.63	..	+0.72	..
With dung	4	8.26	+0.40	..	+0.60	..
Rothamsted:						
No dung	2	8.36	..	+1.64	..	+2.12
With dung	4	7.48	+0.81	+0.87
Light fen:						
No dung	5	6.23	..	+2.49	..	+3.17
Heavy fen:						
No dung	6	10.55	..	+0.19	..	+0.29
Silts:						
With and without dung	4	12.04	..	+0.26	..	+0.24

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THE ROTHAMSTED EXPERIMENTS ON THE MANURING OF POTATOES

PART II. EFFECTS OF INORGANIC AND ORGANIC MANURES ON THE YIELD OF POTATOES

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Effect of Magnesium Salts

IN view of the marked effect of potassium on the growth of potatoes, experiments were made to ascertain whether magnesium, an essential constituent of chlorophyll, would also increase their growth. The question gained additional practical importance from the fact, which came into prominence after the 1914-18 war, that the German potash deposits contain magnesium salts while the Alsatian deposits do not. The salt used was the sulphate so as to avoid complications that might arise from the presence of a chloride.

The early experiments (1921-3) indicated that magnesium sulphate was usually without influence on the growth of potatoes, but might on occasion affect the yield, in some cases beneficially, in others adversely. At Rothamsted an increase was only once recorded; it was on the plots without farm-yard manure but was of doubtful significance. At a few of the outside centres there were marked effects, the most striking being at Blaydon, Durham, where on a light sandy soil the magnesium sulphate improved the size and colour of the foliage.¹ At Newton Abbot (Seale Hayne College) and at Chartley Park on the Bunter Sandstone increases were recorded, but the design of the experiment did not allow the difficulty of soil heterogeneity to be overcome. In a few experiments the magnesium sulphate depressed the yield; in general, however, it had no effect.

The later experiments on modern lines gave very similar results (Table 1). In most cases the magnesium sulphate had no effect. Occasionally, however, it increased the yield, e.g. in 1937, on Bunter Sandstone (p. 218) as in the earlier set, and on a granitic soil; but similar soils gave no increase in 1938, indeed there were two decreases in that season. So far, it has not been possible to account for either the increases or the depressions, but an investigation on the Bunter Sandstone might be fruitful.

Both in the Rothamsted experiments and those of Dr. Cowie sulphate of potash had in most cases given marked increases in yield even where sulphate of magnesium gave none. This result is difficult to understand, as it is in striking contrast with that obtained on Broadbalk Field where sulphate of magnesia was for many years as effective as sulphate of potash and markedly increased the quantity of potassium taken up by the wheat plant, presumably because it displaced potassium from the

¹ *Rothamsted Ann. Rept.*, 1922, p. 19.

soil by a base-exchange process. It is possible that magnesium, while essential in small quantities, may in larger amounts be sufficiently harmful to the potato plant to offset as a rule the benefit of any potassium liberated from the soil.

TABLE 1. *Effect of Sulphate of Magnesium on Yield of Potatoes per acre: Rothamsted and Outside Centres*

<i>No. of expts.</i>	<i>Yield No Mg tons</i>	<i>Increase for Mg tons</i>	<i>Quantity of MgSO₄ applied lb.</i>	<i>Increase for sulph. of pot., tons</i>	<i>Quantity of K₂O applied, cwt.</i>
<i>No farm-yard manure</i>					
Single plots (4) . . .	8.22	-0.39	108	+1.67	1.0
Old-style replicated (5)	8.47	+0.36	172	+2.86	1.0
Modern replicated (Tunstall) (1) . . .	13.05	+0.21	214	+0.76	1.5
	..	±0.41	..	±0.77	..
<i>Farm-yard manure given</i>					
Single plots (3) . . .	10.04	+0.12	101	+1.41	0.9
Old-style replicated (7)	9.20	+0.13	113	+1.05	0.9
Modern replicated (Woburn) (1) . . .	10.90	+0.20	86	+0.10	0.6
	..	±0.28	..	±0.28	..

The earlier experiments include two increased yields by magnesia and one depression which may be significant.

For comparison we have Dr. Cowie's permission to quote his very full set.

<i>Year</i>	<i>No. of centres</i>	<i>Mean yield</i>	<i>Increase for</i>			
			<i>0.3 cwt. MgO</i>	<i>0.6 cwt. MgO</i>	<i>0.7 cwt. K₂O</i>	<i>1.4 cwt. K₂O</i>
1937	24	9.05	+0.07	+0.11	+1.80	+2.17
1938	23	8.57	..	+0.00	..	+2.54

In 1937 there were two increased yields, one on the Bunter Sandstone and the other on a granitic soil, and in 1938 two decreases and one increase, all of which were significant.

Effect of Sodium Salts

One experiment only with sodium chloride has so far been made: it was on newly ploughed grass-land at Rothamsted in 1940. Potash clearly increased the yield, but salt did not, although it did no harm:

	<i>Mean</i>	<i>Responses to mur. pot.</i>	<i>Salt</i>
Total produce, tons per acre . . .	9.02	2.37	0.04
Percentage ware	86.0	5.6	1.2

Effect of Chloride on the Growth of Potatoes

The potato crop differs from several of the others that have been examined, and in particular from sugar-beet, in that the chloride ion is definitely harmful under ordinary conditions. This was tested in several ways: the muriates of ammonia and of potash were used instead of the sulphate; and 'potash salts' containing increasing quantities of sodium and magnesium chlorides (Table 2).

TABLE 2. *Reduction of Yield (tons per acre) by Chlorides: Regression of Yield on Quantity of Chlorine supplied*

Series	No dung		Dung	
	No. of experiments	Mean reduction of yield per cwt. Cl	No. of experiments	Mean reduction per cwt. Cl
Old single-plot .	9	0.17	6	0.21
Old replicated .	13	0.27	27	0.33
Modern factorial	4	0.19	7	0.24

Mean depression in all experiments with and without dung = 0.27 tons.

The chloride depressed the yield in 54 out of 66 experiments, the average reduction being about 5 cwt. potatoes per cwt. chlorine. The depression seemed to be rather more pronounced on light than on heavy soils and greater in the north than at Rothamsted:

	Light soils	Heavy soils	Northern centres*	Rothamsted
Depression per cwt. Cl .	0.38	0.21	0.38	0.13 tons
No. of experiments .	21	22	13	10

* Heavy soils only so as to eliminate soil effect. The period was the same at both groups of centres, viz. 1921-6.

It did not, however, seem to be affected either by nitrogen, potash, or farm-yard manure. The mean depression caused by 1 cwt. Cl in muriate of ammonia was 0.2 ton per acre and that by the same quantity of Cl in muriate of potash was 0.27 ton per acre. The amount of the depression seems to depend on the rainfall: it was least in the wet season and greatest in the moderately dry ones, but lower in the years of exceptional drought both at Rothamsted and at the northern centres (Table 3).

The data are insufficient to allow of positive conclusions, but assuming the result is true it suggests that the chloride ion has two effects on the potato crop: a harmful one shown in all years, which is reduced by high rainfall presumably because the chloride is washed out of the soil; and a beneficial one shown only in very dry conditions.

TABLE 3. *Depression caused by Chloride in Wet and in Dry Seasons*

Years	Rothamsted		Durham centres			
	Mean rainfall inches Jan.-Sept.	Mean depression tons per cwt. Cl	Years	Mean rainfall Jan.-Sept.	Mean depression	
					All soils	Heavy soils
1921 } 1929 } 1923 } 1926 } 1930 } 1931 }	10·8	(4) 0·07	1921 } 1923 } 1922 } 1925 } 1926 }	18·8	(5) 0·18	(2) 0·29
1925	22·2	(1) 0·18	1924	25·0	(3) 0·33	(1) 0·28
1922 } 1924 } 1927 }	25·9	(6) 0·03				

Comparison of Different Potassic Fertilizers

The results in the preceding pages explain and justify the potato-grower's preference for sulphate of potash over other salts. They also explain why the muriate comes second: it is usually cheaper and the depression caused by a 2 cwt. dressing as compared with the return given by the sulphate is of the order of 5 cwt. potatoes per acre, which could not be detected either by observation or by ordinary weighing; indeed we cannot be sure that it is always caused. But for the other potassic fertilizers the depression is clearer: the results of 53 experiments in different parts of the country showed the following response to potassic fertilizers, when the mean response to potash¹ = 100:

	Rothamsted and Woburn			Other centres		
	Sulph. pot.	Mur. pot.	Potash salt 30%	Sulph. pot.	Mur. pot.	Kainit
<i>With dung:</i>						
Cl, cwt. per acre	None	0·96	1·63	None	0·96	3·00
Relative yields .	110	115	76	129	114	57
<i>No dung:</i>						
Cl, cwt. per acre	None	0·78	1·34	None	1·01	3·10
Relative yields .	110	93	98	111	109	80

The Mode of Applying the Artificial Fertilizers

The artificial fertilizers acted better when put into the bouts than if spread broadcast over the field:²

¹ The numbers of experiments were:

	<i>With dung</i>	<i>No dung</i>
Rothamsted and Woburn	4	3
Other centres	27	19

² *Rothamsted Ann. Rept.* 1935, p. 180. The unmanured plot gave 3·9 tons per acre; sulphate of ammonia without dung or potash and phosphate gave no increase.

	Phosphate and potash only		Phosphate, potash, and sulph. amm.	
	Broadcast	In bouts	Broadcast	In bouts
No farm-yard manure .	5.36	5.75	7.26	9.00
Farm-yard manure .	6.78	7.42	7.91	10.41

The 1940 experiment gave similar results, but the differences were smaller.¹

Effect of condition of fertilizers.—In recent years granulated mixed fertilizers have been introduced which are easier to handle than the ordinary material and free from dust so that they can be drilled even on a windy day without loss. Experiments made at three centres on good potato farms show that the granular gave slightly higher yields than the ordinary material, but the difference was not significant (Table 4).

TABLE 4. *Comparison of Granular and Ordinary Fertilizers*

Centre	Soil	Yield: no artificials	Yield with fertilizer*			S.E.
			Ordinary	Granular	Difference	
Wryde, March	Heavy loam	8.77	12.32	12.74	0.42	±0.354
Benwick, March	Good fen	12.02	15.01	15.30	0.29	±0.409
Feltwell, Brandon	Light fen	8.69	10.99	11.00	0.01	±0.727

* Means of 6, 12, and 18 cwt. dressings per acre. No dung used.

Effect of Farm-yard Manure on the Growth of Potatoes

Increase due to farm-yard manure: Value of its nitrogen.—The increase due to a dressing of 15 tons farm-yard manure applied in the bouts has varied round a mean of 2.3 tons per acre,² this being about the increase produced by 0.7 cwt. N or 3½ cwt. sulphate of ammonia. Since the farm-yard manure contained some 0.6 per cent. N, i.e. 1.8 cwt., its nitrogen has about 40 per cent. of the value of that in sulphate of ammonia; this is comparable with the value (50 per cent.) obtained in the Broadbalk wheat experiments for similar quantities of farm-yard manure and sulphate of ammonia.

Reference has already been made in Part I to the remarkable fact that farm-yard manure in spite of its nitrogen-content does not depress the action of the nitrogen in sulphate of ammonia, although it does depress the action of phosphatic and potassic fertilizers. This can equally be stated in the converse form: that sulphate of ammonia has no action on the effectiveness of farm-yard manure, but both potassic and phosphatic

¹ This was combined with a cultivation experiment. Intensive cultivation depressed the yield by 0.66 ton per acre.

² Eleven seasons: the range of increase has been from 1.1 to 3.7 tons.

fertilizers reduce it. This is well illustrated in the 1937¹ experiment at Rothamsted:

Yields of Potatoes, tons per acre

	Sulph. amm. cwt. N			Super. cwt. P ₂ O ₅		Sulph. pot. cwt. K ₂ O	
	0	0.4	0.8	0	0.8	0	1.6
Dung . . .	7.84	9.92	11.06	9.33	9.88	9.42	9.79
No dung . .	4.59	6.44	7.46	5.40	6.92	5.79	6.52
Gain due to dung . .	3.25	3.48	3.60	3.93	2.96	3.63	3.27

The mode of application.—If the manure is spread over the whole field it makes little difference whether this is done in autumn or in spring. The manure must, however, be quickly ploughed under or it rapidly loses value. But it is much more effective to apply the manure in the bouts (Table 5), just before planting the potatoes.

TABLE 5. *Effect of Time and Manner of Application of Dung.*
*Tons of Potatoes per acre**

1934	Dung spread over land and ploughed under					
	In autumn			In spring		
Sulphate of ammonia, cwt. per acre . . .	0	2	4	0	2	4
No dung	9.28	10.06	10.85
Fresh dung	10.47	12.88	13.41	10.66	13.00	13.20
Rotted dung	11.54	12.56	12.88	10.88	12.77	12.74
Increase due to farm-yard manure	1.72	2.66	2.30	1.49	2.83	2.12

	Sulph. amm.	No dung	Dung: 15 tons per acre			
			Ploughed in	In bouts	Increase over no dung	Increase due to dung in bouts over dung ploughed in
1935	7.15	8.06	3.4†	0.91
	1.3‡	..
1936 . . .	0	4.83	6.19	7.92	3.09	1.73
	2	6.13	6.49	8.10	1.97	1.61
1937 . . .	0	4.96	6.02	7.66	2.70	1.64
	2	6.65	7.72	9.76	3.11	2.04
	4	7.73	9.74	11.52	3.79	1.78

* Rothamsted Ann. Repts., 1934, p. 182; 1935, p. 180; 1936, p. 213; 1937, p. 156.

† is mean of all experiments in absence of potash and phosphate, and ‡ is mean when they are present.

¹ Ann. Rept. 1937, p. 156. Fifteen tons dung per acre applied in the bouts.

Increase due to sulph. amm.			
	1936	1937	
Sulph. amm. cwt.	2	2	4
No dung	1.30	1.69	2.77
Dung in bouts	0.18	2.10	3.86

Condition of the dung: fresh and rotted compared.—In a series of experiments lasting three years no difference could be found in effectiveness between fresh and rotted dung. The freshly made dung was given at a standard rate of 15 tons per acre; the rotted dung had been made from equal quantities of similar materials but during rotting had lost one-third of its weight so that the actual dressing was about 10 tons per acre. The rotted dung was richer in N, P, and K than the fresh dung, but the rotting had been of no advantage; on the other hand it had not reduced its effectiveness (Tables 5 and 7).

Possible substitutes for farm-yard manure: the effect of other organic substances.—In view of the marked benefit of farm-yard manure and the difficulties of obtaining sufficient supplies even for the pre-war acreage of potatoes, a series of experiments was begun with possible alternative sources of organic manure.

Straw.—Straw is the most readily obtainable organic material on the farm and its effect has been tested in two ways: (1) chopped and ploughed in at the rate of 2 tons per acre during January; (2) used in proportions heavier than usual for the making of the dung.

The ploughing in of the straw was not a success and usually caused a small decrease in crop (Table 6).

TABLE 6. *Effect of Straw ploughed in on Yield of Potatoes.*
Tons per acre*

	No dung			Dung ploughed in		Dung in bouts	
	Sulph. amm. cwt.	No straw	Increase given by straw	No straw	Increase given by straw	No straw	Increase given by straw
1936 . . .	0	4.83	—0.77	6.19	—0.25	7.92	—0.39
	2	6.13	—0.32	6.49	+0.70	8.10	+1.67
1937 . . .	0	4.96	—0.75	6.02	—0.36	7.66	+0.36
	2	6.65	—0.43	7.72	—0.34	9.76	+0.31
	4	7.73	—0.55	9.74	—0.38	11.52	—0.92
Mean effect of straw	—0.56	..	—0.13	..	+0.20

* *Rothamsted Ann. Repts.*, 1936, p. 213; 1937, p. 156.

The depression was reduced by dung, and, in absence of dung though not always in its presence, by sulphate of ammonia. The dung had residual effects on the succeeding crops, but the straw had not. The direct addition of straw to the land therefore does not seem promising.

The use of larger quantities of straw for the making of the dung was

also ineffective. Two proportions were tested: a 50 per cent. increase in 1938, and 100 per cent. increase in 1939 and 1940. The additional straw did not affect the composition of the final manure as much as might have been expected: the strawy manure was drier than the normal but contained approximately the same percentages of N, P_2O_5 , and K_2O , and after rotting even the nitrogen in the organic matter was approximately the same.¹ But the extra straw contributed nothing to the value of the manure and so far as these experiments go there is nothing to encourage the hope that potato-growers could expand their supplies of farm-yard manure by using more straw in its preparation (Table 7).

TABLE 7. *Comparison of Fresh and Rotted Manure: Effect of Increasing the Amount of Straw on Yield of Potatoes. Tons per acre*

	1938		1939		1940			
	Normal dung	Effect of adding more straw	Normal dung	Effect of adding more straw	Normal dung		Effect of adding more straw	
					(a)	(b)	(a)	(b)
Fresh dung .	13.55	—0.20	11.14	—0.25	8.15	8.90	0.22	0.31
Rotted dung	13.59	—0.11	10.60	0.36	8.77	9.14	—0.16	0.35
No dung	7.23		7.52			

(a) Single dressing.

(b) Double dressing.

Composted straw was inferior to dung (see below).

Residual effects of dung and straw.—A four-course rotation was set up in 1930 to compare the direct and residual effects of dung, composted straw, and straw ploughed in with artificials. The dung was superior to the composted straw in the year of application and in the first year after, but there was no evidence that either of them produced any effect in the later years. Artificials supplying the same amounts of nutrients as the farm-yard manure, but spread over the rotation, gave the best results so long as the phosphate was given as superphosphate. In these experiments the yields have been low for some reason that is not at all clear² (Table 8).

TABLE 8. *Percentages of N, P_2O_5 , and K_2O in the Dung used*

	1938				1940			
	Normal		Strawy		Normal		Strawy	
	Fresh	Rotted	Fresh	Rotted	Fresh	Rotted	Fresh	Rotted
N	0.66	0.75	0.64	0.74	0.62	0.78	0.69	0.93
P_2O_5	0.28	0.37	0.24	0.39	0.27	0.35	0.29	0.42
K_2O	0.82	0.94	0.74	1.06
N in organic matter		3.7	3.9	2.7	4.0

¹ Rothamsted Ann. Repts., 1930, p. 125; 1932, p. 127; 1936, p. 51.

A three-course rotation¹ set up in 1933 confirmed the conclusion that the residual effects from composted straw and from straw ploughed in were small, though in this experiment they were always there. Over the two-year period the effect of the complete artificials was somewhat increased by the addition of straw:

Potatoes: tons per acre

Average 7 years 1934-40	Complete artificials	Straw and artificials ploughed in*		Straw compost
		I	II	
Year of application .	8.50	8.94	8.21	7.10
First year after .	6.36	7.17	7.09	6.77

* In I all artificials are applied in spring; in II half in autumn, half in spring.

TABLE 9. *First-Year Effect and Residual Action of Organic and Inorganic Manures. Four-course Rotation. Rothamsted, average 7 years, 1934-40*

Manure tested	Potatoes (tons per acre)					
	Year of applica- tion	1st year after applica- tion	2nd year after applica- tion	3rd year after applica- tion	4th year after applica- tion	Total of 5 years
Dung,* 16 tons .	4.75	3.98	3.26	3.20	3.50	18.69
Straw compost .	3.51	3.36	3.02	2.78	2.45	15.12
Straw and artifi- cials	4.99	3.66	3.58	3.45	2.92	18.60
Super., 8 cwt. .	5.57	3.96	4.16	3.88	4.03	21.60
Rock phosphate, 4½ cwt. . . .	3.12	2.74	3.48	3.40	2.94	15.68

* Dung and straw compost both supply 50 cwt. organic matter per acre, i.e. sufficient straw is composted (rotted with addition of chemicals) to give 50 cwt. organic matter. All treatments are made up to a total of 1.8 cwt. nitrogen, 1.2 cwt. P_2O_5 , 3.0 cwt. K_2O per acre. In the phosphate series the residues of phosphate only are tested; the nitrogen and potash are applied in five annual doses of 0.36 cwt. N and 0.6 cwt. K_2O , making the standard dressing of 1.8 cwt. N and 3.0 cwt. K_2O in the five-year period. See *Rothamsted Ann. Repts.*, 1932, p. 127, for details.

Poultry and Other Organic Manures

Poultry manure.—In peace-time considerable quantities of poultry manure are available and numerous experiments have been made to find its fertilizer value for different crops. It was used in the dried state, this being the most suitable form for transport, and its composition in the different seasons was not very variable: the average percentages in the samples used were: N 3.6, P_2O_5 3.3, K_2O 1.7. The experiments were

¹ *Rothamsted Ann. Repts.*, 1933, p. 118; 1936, p. 54.

In the three-course rotation the same amount of artificials is used in all four treatments, but in the four-course rotation deduction is made for the nutrients in the straw.

made both at Rothamsted and at outside centres during the period 1933-8: the average results are given in Table 10.

The increase given by the poultry manure is 57 per cent. of that given by sulphate of ammonia supplying the same amount of nitrogen.

TABLE 10. *Comparison of Poultry Manure and Sulphate of Ammonia. Tons Potatoes per acre*

	No. of experiments	Yield No N	Increase for sulph. amm. (0.6 cwt. N per acre)	Increase for poultry manure (0.6 cwt. N per acre)
Light soils .	16	7.74	1.62	1.20
Medium soils .	24	7.18	1.62	0.79
Heavy soils .	14	8.30	1.23	0.64
Mean .	54	7.63	1.52	0.87

Rape dust.—Four experiments have been made with rape dust, in three of which it came out inferior to sulphate of ammonia supplying equal amounts of nitrogen. The average of all the results was:¹

Yield without N	Increase for		Response to N in rape dust when that in sulph. amm. = 100
	Sulph. amm.	Rape dust	
8.00	2.29	1.92	84

Other organic manures.—Single experiments only have been made, but in no case was the organic manure anything like so good as the sulphate of ammonia. The responses to nitrogen in the different substances compared with those for sulphate of ammonia as 100 were:²

<i>Fish meal</i>	<i>Meat meal</i>	<i>Malt culms</i>
43	5	61

¹ Rothamsted Ann. Repts., 1934, p. 210; 1935, p. 212; 1938, pp. 194 and 195.

² Rothamsted Ann. Repts., 1934, p. 210; 1935, p. 212.

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THE ROTHAMSTED EXPERIMENTS ON THE MANURING OF POTATOES

PART III. THE EFFECT OF FERTILIZERS ON THE HABIT OF GROWTH AND OTHER CHARACTERS OF POTATOES. GENERAL SUMMARY

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WITH PLATE 10

IN general, nitrogenous fertilizers in presence of sufficient potash and phosphate cause a marked increase in haulm and in depth of colour of the leaves, a considerable increase in the number of tubers and a lesser increase in their size. Potassic fertilizers lighten the colour of the leaves and keep growth continuing longer in dry seasons; they also increase the size of the tubers. Phosphatic fertilizers used with potash and nitrogen increase the number rather than the size of the tubers.

Plants receiving nitrogen and phosphate without potash may grow quite abnormally, especially in dry conditions. Their leaves become very dark green and some become rather coppery in appearance; the plants are small and growth is restricted. The life of the plant depends on its size: in the drought of 1921 the leaves and stems died earlier than when potash was given, though the yield was not much smaller: in 1940 the growth was so small that the soil moisture sufficed to keep the leaves turgid (Plate 10, figs. 1, 2). The crop given by nitrogen and phosphate without potash was actually less than that without nitrogen:

		P	Increase for
		No N or K	0.6 cwt. N
1940.	No dung .	7.18	-0.50
	Dung . .	8.45	+0.56

Farm-yard manure swamps all these special effects by supplying the complete plant nutrients. On the other hand, town refuse does not always do this, and in 1940 the plants receiving potash and town refuse but no nitrogenous fertilizer were light green and leggy, whilst those receiving nitrogen and town refuse but no potash were very dark green and in parts coppery and stunted; except when the plants were very small they wilted badly in the drought, and they died earlier than those receiving potash in addition. The combination of nitrogen and potash increased the growth of haulms and darkened the colour of the leaves.

Plants receiving no manure at Rothamsted show no special symptoms; they are smaller than the others, but normal in colour and survive as long as any others.

Effect of manuring on susceptibility to insect and fungus attack.—Extended observations were made both at Rothamsted and at Woburn in 1931 by L. M. J. Kramer¹ on the diseases caused by *Phytophthora* spp. (blight) and *Corticium vagum*. The diseases spread from certain centres

¹ Ph.D. Thesis, London Univ.

of infection, but neither these nor the direction of the spread seemed to have anything to do with the manurial treatment: the determining factor seemed to be the direction of the wind. This does not accord with the older idea that moderate manuring decreases the liability to disease whilst excess of any constituent, especially nitrogen or phosphate, increases it. In these particular observations the highest dressing of nitrogen was 3 cwt. sulphate of ammonia, which was clearly not excessive, but no subsequent observations have indicated any clear relation between manuring and incidence of disease.¹

Observations on insect attack have been less systematic, but as far as they have gone they have not shown clear relations with fertilizer treatment.

Effect of manuring on the keeping quality of potatoes in the clamp.—This was tested in 1935.² The average loss of weight in the clamp between mid-October when the potatoes were lifted and February when the clamp was opened was 4 per cent., but with dung in the bouts and complete artificials it was about 6 per cent. Neither sulphate of ammonia, superphosphate, nor sulphate of potash affected the loss appreciably.

About 7 per cent. by weight of the potatoes went bad on storage; dung in the bouts increased this loss to nearly 9 per cent. Sulphate of ammonia had but little effect; superphosphate and potash did not decrease the loss but if anything increased it.

Effect of fertilizers on the size of tubers and the proportion of ware.—Throughout this paper the yield data refer to all the tubers gathered up: ware, i.e. the tubers saleable for food, seed, and chats. The proportions of ware have been ascertained in most of the experiments: they are not strictly comparable for all years as the size of the riddle over which the potatoes must pass is specified from time to time by the Potato Marketing Board. But they are comparable in any one year for the different plots, and so the results show how the proportion of ware is affected by the fertilizer treatment or the soil conditions.

The results up to and including 1936 have already been discussed in detail by one of us,³ and we therefore give here only a summary including the more recent data.

On unmanured land the tubers tend to be small, and only 50 or 60 per cent. may be large enough to be sold as ware. Manuring markedly increases the size. There is a general relation between the yield and the size of the tubers, and in comparable experiments the percentage of ware increases with the yield.⁴ But the effect of individual fertilizers is not quite the same on size as on yield. In most of the experiments sulphate of ammonia has increased the yield, but in only about half of them did it raise the percentage of ware, and then only by a small amount excepting where the initial percentage of ware was low. Superphosphate also increased the yield more often than it increased the ware. On the other

¹ At Susworth (Lindsay), 1939, the percentage of diseased tubers was:

0	1	2	3	cwt. sulphate of potash per acre
1.2	1.4	1.5	0.8	per cent. blighted tubers

² Rothamsted Ann. Rept., 1935, p. 181.

³ H. V. Garner, this Journal, 1937, 5, 327-41.

⁴ Loc. cit., p. 330, fig. 1.

hand, potash markedly increased the proportion of ware, and farm-yard manure still more. In all cases the effects are more marked when the initial percentage of ware is low, e.g. around 50 per cent.: where it is already above 90 per cent. fertilizers have no effect.

The effects are summarized in Fig. 3.

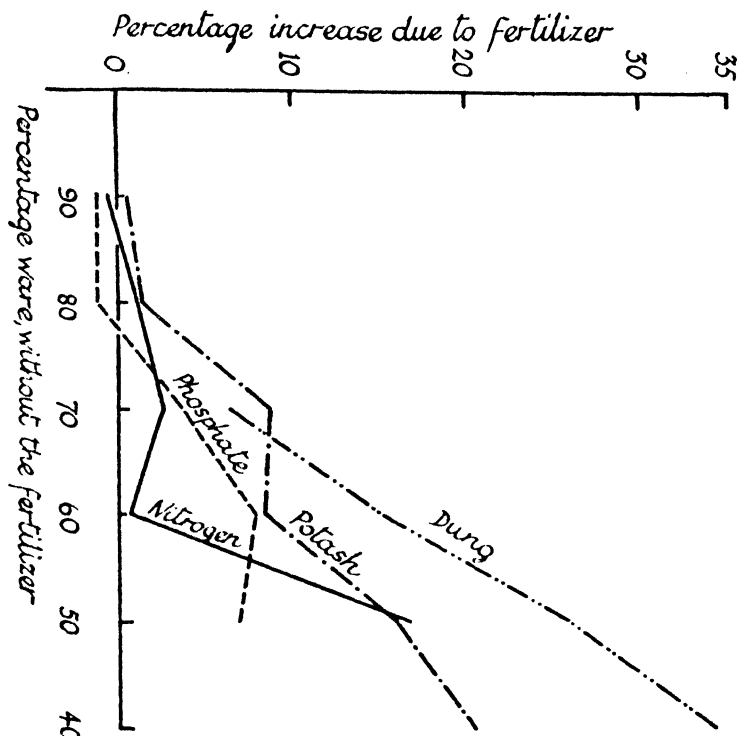


FIG. 3. Influence of manuring on the percentage of ware, i.e. the size of the tubers.

As in the case of yield the effect of farm-yard manure was somewhat greater when it was applied in the bouts than when spread over the whole field, and it was diminished by potassic fertilizer. The further addition of straw had no effect on the proportion of ware.¹

Summary of Effects of Fertilizers on the Growth of Potatoes

Potatoes differ from most farm crops in responding freely to all three groups of fertilizers, and not chiefly to one or two, as often happens. The response to sulphate of ammonia is high: about 16 to 20 cwt. of potatoes containing about 320 to 400 lb. starch equivalent can be obtained per cwt. of sulphate of ammonia provided potash and phosphate are sufficiently supplied; this is more starch equivalent than can be obtained from any other crop for the same quantity of nitrogen. The response is not much affected by the soil type (except on the fens), the soil condition, the presence or absence of farm-yard manure, or the magnitude of the basal yield; it is the least variable of any of the fertilizer responses. But

¹ *Rothamsted Ann. Rept.*, 1937, p. 155.

it varies with the size of the dressing; the increase per unit of nitrogen is less for higher than for lower dressings; nevertheless, the rate of fall is slow and we have continued to obtain good increases for even the third or fourth hundredweight of sulphate of ammonia per acre. The full effectiveness of the sulphate of ammonia is obtained only when adequate phosphate and potash are present, and the relation with phosphate is more marked than that with potash, except on heavy soils. The nitrogenous fertilizer increases the yield partly by increasing the size of the tubers and partly by increasing their numbers, but except in poor soil conditions the chief effect appears to be the increase in number. There is no clear evidence that nitrogenous fertilizers affect the susceptibility of the leaves to disease.

The increases due to superphosphate may be even greater than those due to nitrogen, but they are not so widespread; 5 cwt. superphosphate has given responses of 3 tons or more per acre, though a more common increase is about 1 to 1½ tons per acre. The response differs from that to nitrogen in several ways: it usually falls off more steeply at the higher doses; it is more variable and more affected by soil and other conditions. Thus it is greater on heavy than on light soils; it varies on soils of different degrees of productiveness; on the heavy soil at Rothamsted it tends to be greater in years of low than of high yields, but this does not hold for the light soil at Woburn. The response is depressed by farm-yard manure. On the other hand, it resembles the response to nitrogen in that it is enhanced by potash.

Like nitrogen, phosphate increases both the size and the number of the tubers, probably the number more than the size. The combination of phosphate with excess of nitrogen and no potash may lead to harmful effects (p. 227); this does not often happen in practice, though G. A. Cowie has observed it in some of his experiments.

The response to potash differs from that to nitrogen and resembles that to phosphate in its rapid fall at higher doses, in its variability, and in its depression by dung. It differs from both nitrogen and phosphate responses in being more closely connected with the productiveness of the soil and the season, and is greater in poor conditions. It resembles both responses in being enhanced by the presence of the other two nutrients. Except on the fens there is no evidence that light soils are more responsive to potash than heavy ones.

The effect of potassic fertilizers on the size of the tubers is more pronounced than that of nitrogen or phosphate, and this may indeed be the chief cause of the increase in crop.

Potatoes differ sharply from mangolds and sugar-beet in that they are adversely affected by chloride; in our experiments 1 cwt. Cl per acre has reduced the crop by about 5 cwt. per acre. It is not clear whether sodium is harmful, though there is no evidence that it is beneficial. Magnesium in general is ineffective, but on a few soils, e.g. the Bunter sandstone, it has been beneficial. These results justify the growers' preference for sulphate of potash.

The fertilizers acted better when applied in the bouts than when broadcast.

No evidence could be obtained that manuring appreciably affected the liability to disease or the keeping quality.

The effect of soil type on yield is less than might have been expected in view of the marked preference shown by growers for light soils and loams. The determining factor is the ease of cultivation and especially of lifting, which is much greater on light than on heavy soils.

Farm-yard manure has a special value for potatoes, increasing both the yield and the percentage of ware, and the plots at Rothamsted and Woburn from which it is regularly omitted give only poor yields. Even on the fields which periodically receive dung its omission from the potato crop has lowered the yield by some 3 tons per acre. As already stated, farm-yard manure does not lower the effectiveness of sulphate of ammonia though it reduces that of phosphate and potash. The farm-yard manure is more effective when put into the bouts in spring than when spread over the whole field and ploughed under.

Rotting was no advantage: 15 tons of freshly made dung gave the same results as the 10 tons of rotted dung which it yielded after storing.

Straw composts were not as effective as dung.

The responses to the standard dressings of fertilizers are set out in Table 1. These are the increases over the means of all other treatments, and as shown in Part I, they are lower than if the comparison had been confined to plots fully supplied with the other nutrients, as would happen in good practice. But they may more nearly represent the conditions of ordinary practice.

The figures fall mainly on to simple distribution-curves. There are some apparent decreases, almost entirely in the range 0–10 cwt. per acre, but as the significant difference in an experiment is rarely less than 8 cwt. per acre,¹ a certain number of these figures simply mean that the fertilizer had no effect.

In absence of dung the responses to nitrogen are fairly widely distributed, about half of the increases are between 0 and 20 cwt., but some are as much as 40 cwt. of potatoes for 0.25 cwt. nitrogen, representing a recovery of some 65 per cent. or more in the crop. The results for phosphate are also widely distributed, but those for potash are peculiar in that they fall into two groups: most of the soils gave increases varying from 0 to 30 cwt., but a certain number form a special group giving increases of over 60 cwt. potatoes for 0.5 cwt. K_2O —representing an even higher recovery than for nitrogen.

In presence of dung the responses are all telescoped; the distribution of the increases is nothing like as wide; most are clustered round the groups 0 to 20 cwt. and few reach 40 cwt. The increases for potash are practically all in the group 0 to 30 cwt., and there is no sign of a second and higher group. This telescoping of the potash and phosphate responses can be attributed to the depressing action of farm-yard manure on their effectiveness, but the telescoping of the nitrogen effect cannot be so explained as there is no depression in this case. There were many more experiments without than with dung and this may account for the apparent discrepancy.

¹ H. V. Garner and J. W. Weil, this Journal, 1939, 7, 369.

TABLE I. *Numbers of Responses of the Specified Magnitudes to the Standard Dressings*(0.25 cwt. N, 0.5 cwt. P_2O_5 , 0.5 cwt. K_2O)

	Decrease		Increase							
	20 to 10	10 to 0	0 to 10	10 to 20	20 to 30	30 to 40	40 to 50	50 to 60	Over 60	
Magnitude of response, cwt. per acre										
No DUNG										
Nitrogen:										
Mineral soils, light and medium . .	1	6	16	26	11	10	5	1	1	
" " heavy	1	11	9	8	4	2	
Fen soils, light	2	5	3	3	
" " heavy	3	4	..	1	..	
Phosphate:										
Mineral soils, light and medium . .	1	8	6	6	6	1	1	
" " heavy	1	2	3	3	..	1	
Fen soils, light	4	2	3	1	
" " heavy	2	1	1	3	1	1	
Potash:										
Mineral soils, light and medium . .	1	4	13	8	5	1	1	1	3	
" " heavy	2	6	3	5	2	2	..	3	
Fen soils, light	3	2	1	3	1	2	3	
" " heavy	2	6	
WITH DUNG										
Nitrogen:										
Mineral soils, light and medium	4	12	5	2	3	2	
" " heavy	2	6	4	6	4	1	..	1	
Fen soils, light	1	5	1	
" " heavy	3	
Phosphate:										
Mineral soils, light and medium	5	9	..	2	1	
" " heavy	2	4	
Fen soils, light	1	4	2	1	
" " heavy	2	
Potash:										
Mineral soils, light and medium	2	8	10	4	2	1	
" " heavy	2	8	8	6	1	1	
Fen soils, light	2	..	2	2	1	
" " heavy	1	1	

Note: The responses are averages over all other treatments in the case of the recent factorial experiments.

The final results are collected in Table 2, where also are set out the averages obtained by E. M. Crowther and F. Yates for all the experiments made in Great Britain since 1900 to which they could obtain access.¹ As with other crops the Rothamsted average results are fairly close to the average for Great Britain.

¹ E. M. Crowther and F. Yates, this Journal, 1941, 9, 77-97.

TABLE 2. *Summary of Fertilizer Effects on Potatoes*

	N (0.25 cwt. per acre)		P ₂ O ₅ (0.5 cwt. per acre)		K ₂ O (0.5 cwt. per acre)	
	No dung	Dung	No dung	Dung	No dung	Dung
All centres . . .	0.96	0.82	0.87	0.44	1.11	0.71
Rothamsted . . .	1.11	1.16	0.88	0.61	1.76	0.45
Great Britain . .	1.07	0.86	0.84	0.55	1.23	0.55

Response to 10 tons farm-yard manure

	Dung alone	Dung in presence of artificial
All centres	2.6	1.3
Rothamsted	3.0	1.4
Great Britain . . .	2.8	1.4

The quantities of the dressings are arbitrary; if the effects are reduced to those produced by quantities proportional to the atomic weights of the three nutrients they become, for Rothamsted and its outside centres:

	N	P	K
Cwt. per acre	0.14	0.31	0.39
Response, tons potatoes .	0.61	1.05	1.08

Effect of increasing dressings of a complete fertilizer.—The effect of increasing the dressings of mixed fertilizer of suitable composition was studied at several centres. At all except one the yield increased with the dressing, and on the fen and heavy loams the increase was substantial even up to the 18 cwt. dressing; no dung was given. On the Lincolnshire limestone soils the level of yield was low and the increments were somewhat less, though in absence of dung they still continued up to the 12 cwt. dressing. (Table 3.)

TABLE 3. *Increasing Levels of Complete Fertilizer*
Yield of potatoes (tons per acre)

Centre	Soil	Yield: no artificial	Increase for fertilizer mixture cwt. per acre						
			4	6	8	12	16	18	
Midland College (5 expts. 1934-9)	Good light loam	9.64	0.56	..	0.35	0.31	Dung
Lincolnshire . . (5 expts. 1935-7)	Limestone	5.67	0.97	..	1.92	2.35	One with, four with- out dung
Three good potato farms (1939) . .	2 fens; 1 heavy loam	9.83	..	1.91	..	3.25	..	4.03	No dung

Manurial Practice

The recommendations for the manuring of potatoes have varied somewhat since the time when Augustus Voelcker made his first experiments

seventy years ago.¹ He then used a mixture of approximate composition $N:P_2O_5:K_2O = 1:1:1$, but gives no reasons for his choice. Lawes and Gilbert used more potash than phosphate. Then came a period when the proportion of phosphate was increased. A. D. Hall in 1909 suggested $N:P_2O_5:K_2O = 1:2.5:2.5$. Many of the 'compounds' sold to farmers, however, contained less potash. The more recent work at Rothamsted indicated that more nitrogen and more potash should be used, and the proportions suggested before the war were $1:1:1.2$.

	Voelcker 1870	Lawes and Gilbert 1883	Good practice 1900		A. D. Hall 1909	Rothamsted	
						Usual	Heavy fen
			(a)	(b)	(c)		
N .	1	1	1	1	1	1	1
P_2O_5 .	1	0.7	1	1.1.5	2.5	1	2.3
K_2O .	1	1.6	0.8	1.5	2.5	1.2	1

(a) A well-known manure dealer. (b) Primrose McConnell, *Agricultural Notebook*. (c) *Fertilizers and Manures*, 1909.

The Rothamsted combination is based on the experiments described in this paper, but it is intended only as a framework to be modified according to conditions. Farm-yard manure should be the basis of the manuring; 15 tons per acre may give 2 or 3 extra tons of potatoes. In view of the good response to sulphate of ammonia and the fact that it goes on to the third and sometimes the fourth hundredweight per acre and is not curtailed by farm-yard manure, we suggest that 3 cwt. should be the normal dressing, to be increased to 4 cwt. if the other conditions allow of yields of the order of 9 or 10 tons per acre. The dressing of superphosphate must depend partly on the soil; heavy soils being more responsive can have 4 cwt. per acre, and heavy fen soils 5 cwt. or even more. The yield does not continue to rise with the higher dressings quite as well as with sulphate of ammonia, but the increments may still be profitable. Light soils may be less responsive and 3 cwt. may suffice, though higher dressings should be tested. About potash it is more difficult to generalize: 2 cwt. sulphate of potash could be taken as a normal level, to be increased if supplies of dung are short, or decreased if they are larger or the soil is in a condition of high productiveness. As between the sulphate and the muriate of potash there is little to choose, but the lower-grade salts may involve loss of crop, as their chlorides reduce the yield and there is no evidence that either sodium or magnesium normally increases it.

It is recognized that in war-time the quantities of potash involved in this recommendation are not available. If all potato-growers were equally good the most economical use of restricted potash supply would be on the soils in poor condition, as it is these which usually give the greatest response. But there are other factors besides soil condition, and the rationing of potash involves a variety of considerations.

¹ A. Voelcker, *J. Roy. Agric. Soc. Eng.*, 1870, 6, 392.

Manuring of ploughed-out grass-land.—Potatoes can very suitably be grown as a first crop on newly ploughed grass-land. At Rothamsted the need for potash is as great, and for nitrogen almost as great, as on the arable land, indicating that the grass residues supply only small amounts of nitrogen and potash to the crop. The response to phosphates is somewhat less than on the arable land, but this should not be regarded as generally true.

TABLE 4. *Fertilizer Response on Ploughed-out Grass and on Old Arable Land. Rothamsted, 1939-41*

The responses are calculated to the standard dressings
N 0.25 cwt., P_2O_5 0.5 cwt., K_2O 0.5 cwt.

<i>Ploughed-out grass</i>						
<i>Fertilizer applied</i>	1939		1940		1941	
	<i>Basal</i>	<i>In-crease</i>	<i>Basal</i>	<i>In-crease</i>	<i>Basal</i>	<i>In-crease</i>
0.25 cwt. N	8.58	+0.52	8.51	+0.17
0.5 cwt. P_2O_5 .	6.89	+0.58	8.83	+0.34	8.43	+0.32
0.5 cwt. K_2O .	6.85	+0.63	7.84	+1.90	7.75	+1.67
<i>Old arable</i>						
0.25 cwt. N	7.32	+0.24	4.28	+0.88
0.5 cwt. P_2O_5 .	6.29	+1.47	4.43	+0.59
0.5 cwt. K_2O .	6.40	+1.05	6.76	+1.10	3.88	+1.69

No dung was given in any of these experiments.

(Received June 7, 1941)



FIG. 1. Effect of absence of potash in year of exceptional drought. (Jan.-Sept. 10.94 inches.)
No dung given. Left: N, P, K. Right: N, P.

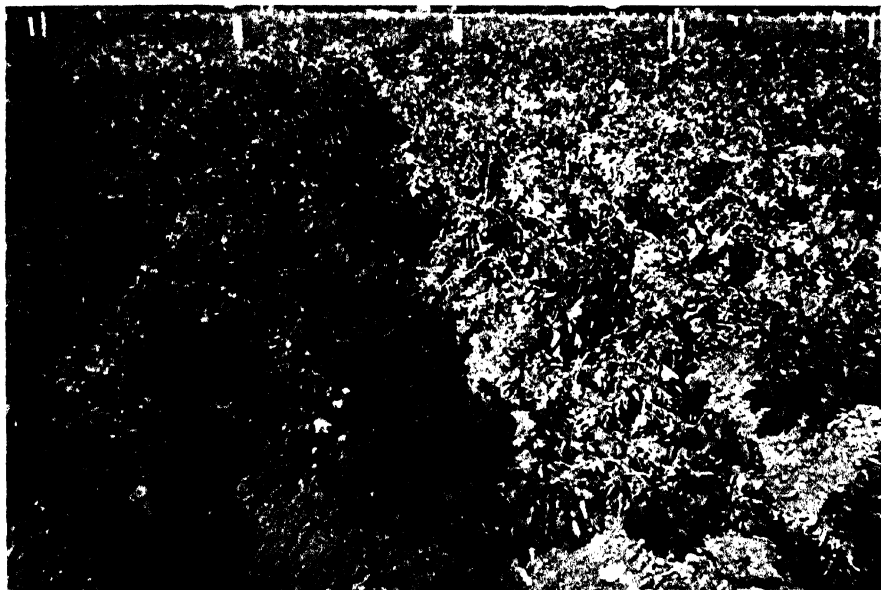


FIG. 2. Foliage-effects in year of moderate rainfall. (Jan.-Sept. 17.11 inches.) No dung given.
Left: NP very dark green. Right: PK very light green.

MANURING *HEVEA*. III. RESULTS ON YOUNG BUDDINGS IN BRITISH MALAYA

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WITH PLATES 10, 11

Summary.—The results of fourteen manuring experiments with young budded rubber on the inland soils of British Malaya are described. Phosphate has proved to be the outstanding stimulant of growth, earlier maturity to the extent of between 6 months and 2 years being easily gained when manuring was started in good time. Nitrogen gave much smaller effects and potash normally gave nothing measurable. Interactions between the three elements were not of great importance. The yield comparisons as yet refer only to early tapping years, but effects are signally small compared with the known influences on vigour and growth.

The experiments.—The manuring experiments under review were laid down on the Dunlop rubber estates in 1934 in collaboration with the staffs of the Rothamsted Experimental Station and the Agricultural Research Department of I.C.I. Ltd. (Jealott's Hill). The series is confined to stands budded with modern clones, and comprises nine experiments on trees which were immature at the start, being 2 years from budding, with five experiments on trees which were nearing maturity.

No safe assumptions could be made in designing the experiments as to the forms and optimal rates of fertilizers to be used, although it was known that there was much room for improvement in growth-rates. Experience with older stands has also shown that at some stage in the productive life it is necessary to manure (chiefly with nitrogen) if deterioration is to be avoided. The design chosen was therefore the 27-plot layout in which nitrogen, phosphate, and potash at three levels (0, 1, 2) are used in every possible combination. The Technical Communication No. 35 of the Imperial Bureau of Soil Science, by F. Yates, may be consulted for the analysis of this layout, and it will suffice to say here that the main effects of each element in two doses can be assessed from averages of 9 plots (since out of the 27 plots we can pick for each element 9 controls with nothing, 9 which receive the unit dressing, and 9 for double dressing), whilst first-order interactions between pairs of elements can be assessed from averages of threes. The design also allows a single replication of 27 plots to be set out in 3 blocks, the standard error being estimated from the interaction terms other than the linear first-order ones. In accordance with a convention adopted by Yates, the interactions are given as one-half the difference in response to one fertilizer in the presence and absence of a second.

Throughout the paper degrees of significance are indicated by ** for P less than 0.01, and by * for $P = 0.01$ to 0.05.

For most of the immature stands the layout consists of 5 rows of trees half a mile long to each treatment, of which the middle three only are used for measurements. Each treatment is separated from the next by one unmanured row, so that the plots of 3 experimental rows alternate with plots of 3 guard rows, and each plot of 3 experimental rows is thus protected by similarly treated neighbouring rows. The original intention was that each plot of 3 rows would form a complete commercial task for recording of yield, and their size and shape were selected on that basis, but new methods of recording by sampling of trees with cup-coagulation have since been introduced, and the tasking now crosses the plots at right angles, so reducing the error due to variation of tapping skill. This method of recording permits latitude in the plot-size.

From considerations of the statistics gathered and the similarity of the responses in the individual experiments, it was found feasible in 1939 to reduce the plots to one-third of their former length without much loss of information.

On one estate of immature rubber 13,000 acres in extent, planted in 1930, at spacing 12×18 ft., budded 1931-2, nearly one-tenth of the total area was laid down to these experiments. The remaining five 'mature' experiments were laid down on two other estates, also on a task basis, these being of a more compact form, as commercial tasking had already been done. Guard plots separate the manured plots from one another. These experiments have since been re-tasked so as to split up each plot between a number of tappers, and the plot-size reduced to 180-200 trees.

The following approximate figures indicate the scale of the undertaking:

Acreage of plots (excluding guards)	1,260
Tons of fertilizer used in 1938	317
Number of girths measured	161,000
Trees sampled for yield	90,000
Standard analyses of variance	350

Soil.—The soil of the immature group is a harsh white clay derived from quartzitic shales. Large sections must have been subject to frequent flooding under its original jungle conditions, and lateritic gravel occurs in all slightly elevated areas. In Table 1 are given the analysis figures for a composite sample, which sufficiently indicates the soil type.

Clones.—The experiments with mature trees include only two clones Av. 50 and Av. 49. Those with immature trees, which were planted later, represent a wider choice and include 3 experiments with B.D. 5, 2 with P.B. 23, and 1 each with Av. 256, P.B. 186, Tj. 1, G. 1.

Rates of application.—Table 2 gives the rates of application originally specified, which have so far been adhered to. To assist in application, the mixtures were machine-mixed, delivered in 100-lb. bags for easy man-handling over rough ground, and doled out to each tree by the aid of galvanized iron mugs, specially made to the requisite sizes for the different plots. Weeded rentices down the rows are used for application, which is accompanied only by very light surface-cultivation or scuffling.

TABLE 1. *Soil Analysis*¹

Coarse sand . . .	16.2 per cent.	'Available' P ₂ O ₅ (Truog's method) . . .	3.8 p.p.m.
Fine sand . . .	34.7 "	Total P ₂ O ₅ (HCl-soluble) . . .	100 "
Silt . . .	26.3 "	'Available' K ₂ O (Milne's method) . . .	71 "
Clay . . .	23.0 "	Total K ₂ O (HCl-soluble) . . .	660 "
Moisture . . .	1.44 "	Nitrogen . . .	700 "
Sticky point . . .	21 "	pH . . .	4.06
		Conductivity (1:5 water extract) . . .	23 × 10 ⁻⁶ mho.

TABLE 2. *Unit Rates of Application of Fertilizers in ounces per tree*

<i>Age since budding, years</i>	2	3	4	5	6 and over
1N. Sulphate of ammonia	10.0	13.4	16.0	18.7	21.4
1P. Cheribon rock phosphate*	7.2	9.0	10.8	12.6	14.4
1K. Sulphate of potash	4.5	5.6	6.7	7.8	9.0

* Cheribon phosphate, 28 per cent. total P₂O₅ (13 per cent. citric-soluble P₂O₅), used in 1934-5-6, Christmas Island phosphate 36 per cent. total P₂O₅ (11 per cent. citric-soluble P₂O₅), used in 1937 onwards, reduced in amount to give the same total P₂O₅. The logical possibility that the fading of the phosphate effect after 1936 was due to the change in source of supply or dosage is negated by the results shown later in Table 10.

GROWTH DATA

Experiments on immature trees.—Girth measurements have been taken annually (in a few cases half-yearly) on a sample of trees selected as 1 in 10 and permanently marked. The first cardinal tree in each row was selected at random and every tenth one was marked off from this. No apology for retaining a fixed sample of trees will be required when it is realized that, since growth-rates or increments derived as girth-differences are highly desirable for analysis, each sample of trees has to be measured twice, and to take a new sample each time would double the amount of work, besides adding greatly to the complication of tree identification. Marking of all trees with permanent numbers is not feasible when they are small, although this was finally carried out at a later stage. This fixed sample of trees differs slightly in character from the final stand by reason of a small class of late buddings or supplies which grew up after the selection was made. The effect of omitting these small trees was proved by additional work on new samples in 1939 to give at that date average girths 10 per cent. in excess of actual. The manuring effects of nitrogen and phosphate had also been over-assessed by about 0.8 cm., since the small class, as late arrivals, are repressed by overshadowing, and this is exaggerated by manuring, so giving more relative weight to the class when introduced into the sample.

The original stand at early stages has an abnormal size-variation due to the succession of budding rounds, but it may be noted that increments or growth-rates are more uniform, for if the cambium lays down wood at the same rate on all trees, the girth-increments are the same on

¹ The authors are indebted to the Soils Division, Rubber Research Institute of Malaya, for these figures.

small late-budded trees as on larger early-budded ones. This applies to such an extent that average increments are subject to a very much smaller error than average girths during the early development of a budded stand, and so are more sensitive and reliable for the proof of experimental responses.

MAIN RESULTS

The general nature of the results and method of presenting the data for this layout can best be exemplified by giving the average girth-figures in the 1938 campaign of measurement for the immature group. They represent the effects after rather more than four years' action of the fertilizers. The primary data were entered as 243 values (9 experiments by 27 treatments), each being the average of a sample of 50 to 60 trees. Table 3 sets out the 27 averages for treatments, the 3×3 tables for studying interactions, the final summary of results, and analysis of variance. The average girth of the trees when they began was between 11 cm. and 15 cm., so that about three-quarters of the total growth measured in the Table may be reckoned to have taken place while under the influence of the treatments.

The major effect is produced by phosphate, an average extra girth of 8 cm. being attributable to the double dressing, which corresponds to maturity being advanced by a period approaching two years. The unit dressing produces significantly more than half the effect of the double dressing, giving sufficiently reliable data from which to attempt an estimate of the optimum dosage when questions of outlay and return are properly introduced. One such calculation gave a return from double phosphate valued at five times its cost, from which it can be derived that an optimum dressing for the period would be in the region of $2\frac{1}{2}$ times the unit chosen for the experiments. Nitrogen has produced a smaller (but still significant) effect—about one-third that of phosphate. Since the cost of the nitrogen treatment is three times that of phosphate, its effects are some nine times as costly to produce, and would scarcely seem economic, though a similar estimate to that for phosphate indicates that a low dose of about $\frac{1}{2}$ unit might just be profitable. The effects of potash are very small indeed and not significant. The only important positive effect of potash on record in these experiments was a 25 per cent. assistance to growth during recovery (Expt. No. 10) from heavy wind damage which occurred at the end of two years' treatment, when a large proportion of trees were pollarded. It is important to notice that none of the interactions assume significant values in these average effects. This provides reliable evidence, where evidence was previously scanty, on the question of providing balance in fertilizers for rubber. Interdependence has not been important.

The manner in which the effects have developed has points of special interest and these are depicted in Fig. 1, where the extra girths gained as the main effects of nitrogen and phosphate are plotted against time. No correction has been made for the small interactions, and the potash effect has been passed over, as it cannot be said to exist. The figure has been derived from six experiments (three being omitted because of

TABLE 3. *Girths of Immature Trees after 4½ years' Action of Fertilizers*
Average of nine experiments. General Mean 47.17 cm.Primary Table. Average for each of $3 \times 3 \times 3$ treatments. S.E. ± 0.632 .

	oK oP	oK 1P	oK 2P	1K oP	1K 1P	1K 2P	2K oP	2K 1P	2K 2P
oN	41.5	47.1	48.1	40.1	47.1	49.2	42.1	46.2	49.4
1N	42.2	47.9	51.0	42.3	49.7	50.3	42.6	49.5	52.0
2N	43.7	49.2	52.6	43.8	50.1	51.4	43.7	49.4	50.9

Two-Factor Tables. Averaging third factor. S.E. ± 0.366 .

	Effect of N and P averaging K			Effect of P and K averaging N				Effect of N and K averaging P			
	oN	1N	2N		oP	1P	2P		oK	1K	2K
oP	41·5	42·3	43·7	oK	42·5	48·1	50·5	oN	45·6	45·7	45·9
1P	46·8	49·0	49·6	1K	42·3	48·9	50·3	1N	47·0	47·4	48·0
2P	48·9	51·1	51·6	2K	42·8	48·4	50·8	2N	48·5	48·4	48·0

Linear Interactions: one-half the difference in the responses to the double dressing of one fertilizer in the presence and absence of the double dressing of another, e.g.

$$N \times P = \frac{1}{2} \{ (2N.2P - 2N.oP) - (oN.2P - oN.oP) \}.$$

$$N \times P + 0.25 \quad P \times K - 0.05 \quad N \times K - 0.37 \quad \text{S.E. } \pm 0.366.$$

Single-Factor Tables. Averaging other two factors.

Means					Responses			
	N	P	K		First dressing (1-0)	Second dressing (2-1)	Linear responses (2-1)+(1-0)	Curvature (2-1)-(1-0)
0	45.7	42.5	47.0	N	+1.78**	+0.82*	+2.60**	-0.96
1	47.5	48.5	47.2	P	+5.96**	+2.06**	+8.02**	-3.90**
2	48.3	50.5	47.3	K	+0.15	+0.13	+0.28	-0.03
	S.E. ± 0.211			S.E.	± 0.298	± 0.298	± 0.298	± 0.519

Analysis of Variance of 9×27 Table

	D.F.	Mean square
Experiments (Clone and site)	8	338.1
Blocks within experiments	18	22.7
Treatment: Linear responses	3	960.3
Curvatures	3	72.3
Interactions	3	1.8
Treatments \times experiments	72	4.9
Remainder	135	3.596
Total	242	..

Standard error per plot ± 1.896 .

interrupted history) by plotting growth-curves from the field data, then reading off and averaging the values at six-monthly intervals. The

nitrogen effect developed normally and steadily, whilst the phosphate effect, after suffering a short period of induction at the start, passed on to a very rapid development and then slowed down again as though approaching a stage of saturation. These changes correspond exactly to a wave of growth lasting about three years and reaching its maximum in 1936, about four years from budding. Curves of growth-rates for *oP* and *2P* plots are introduced into Fig. 1 to show this. The *oP* curve may

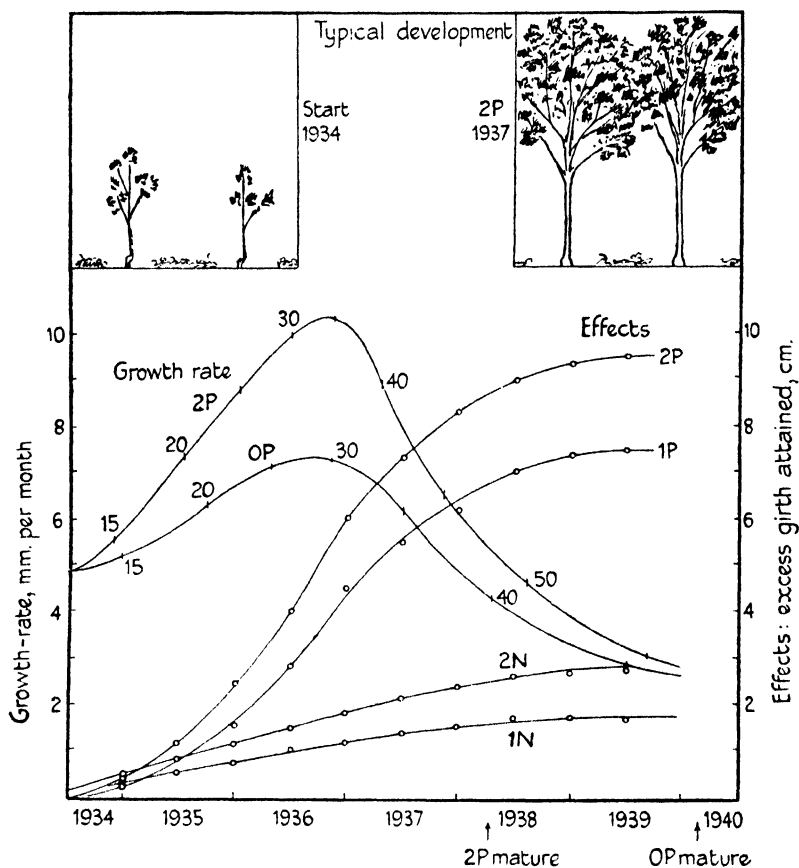


FIG. 1. Mean effects of N and P on girth and growth-rate, average of six experiments. The strokes on the growth-rate curves mark the dates at which the girth reached 15, 20, 25, &c., cm.

be taken as a characteristic or control rate-of-growth curve for the period. The common origin of the two curves at about 5 mm. per month is simply the average rate from budding, revealed by the first girth-measurements. Over the first two years the *oP* rate increased to a maximum of about 7 mm. in 1936 and then fell to about half this value a year and more before tappable size was reached. That means that the final steps to maturity are very slow. Under the stimulation of phosphate the *2P* plots reached a maximum rate of over 10 mm. per month ($4\frac{1}{4}$ in.

per year). On a good site that rate would be a good one without manuring for any immature stage. In spite of the stimulation shown by manuring, the same fall in rate set in without regard to clone or treatment, so that *2P* plots had slowed down to $4\frac{1}{2}$ mm. per month by the time they reached maturity. It seems probable that such a period of maximum stem-growth, corresponding to the autocatalytic curve as roots and leaves multiply, may always be expected. In this special instance the feature was exaggerated, firstly, by the very poor start the planting had, and secondly, by an unusually rapid and severe onset of competition between trees, due to poor and shallow soil. However that may be, the changes in rate of growth are a basic feature of the data before us. The difficult thing to explain is the fact that, although certain clones and certain treatments were much earlier than others in branching and closing their canopy, this had no appreciable effect in shifting the time of maximum growth or the fall we attribute to competition. In the diagram the times are marked at which *oP* and *2P* plots reached certain average girth-values, and we see that the particular stage from 30 to 35 cm., for instance, was passed through by the *2P* plots before reaching the zenith

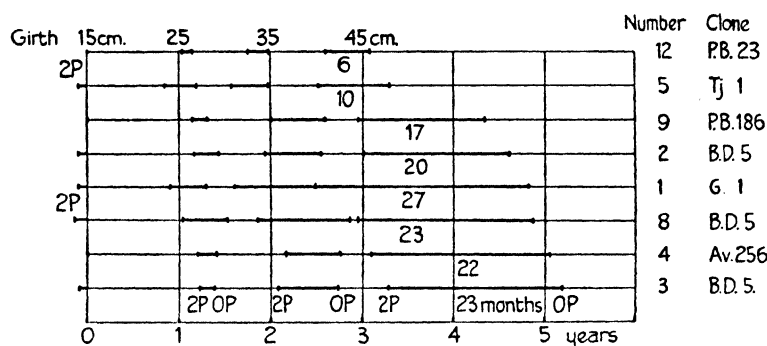


FIG. 2. Comparison of times taken to reach various girths for treatments *oP* and *2P* in individual experiments.

of activity, whereas the *oP* plots passed through it after passing the zenith. The wave depended on the age (or the date) and not upon the stage of development as measured by size of tree. The question of rain-fall was examined without revealing any likely explanation in that direction. The plain practical conclusion is that early manuring with phosphate in such a case as this secured special advantages for big responses, and that the opportunity is comparatively restricted after the passing of the fifth year from budding. Phosphate has been specially associated in some way with stimulating site-exploitation by the tree during its immature phase. Simple considerations of rate of root-growth fail to provide a full explanation because the onset of competition did not come much earlier in the plots of greatest stimulation.

Time gained.—The advantages gained by way of earlier maturity from the use of phosphate are brought out in Fig. 2, where the individual experiments are compared. This diagram shows a time-scale whose zero for all experiments has been made to correspond to the date (early

in the experiments) at which *oP* plots reached an average girth of 15 cm. The times are then marked at which the *oP* and *2P* plots reached averages of 25, 35, and 45 cm., and the lengths of the thick lines between such points represent the times gained at the particular stages of growth. The experiments are placed in the order in which the *oP* plots reached the last value, thus grading them upon a factor combining the clone vigour and the site-fertility. The time gained at 45 cm., which is a little before tapping begins, varies from 6 months for P.B. 23, a strong clone on one of the better sites, to 27 months for G. 1. The latter is undoubtedly one of the poorer sites, but with the use of phosphate this clone did as well as the very vigorous clone Tj. 1.

Trees can be opened for tapping when a girth of 20 in. is reached, and maturity of a stand is defined as 70 such trees existing per acre. This corresponds to an average girth of about 47.5 cm., and the dates of maturity for this standard have been marked by arrows in Fig. 1 for *oP* and *2P* plots (without correcting for interactions). The average advance in the tapping-date due to the double phosphate dressing is 22 months.

Tappable stand at opening.—The present chapter in the story of these experiments has been closed by tapping. An excellent index of the final position is afforded by the number of trees per plot brought into tapping, a summary of analyses being given in Table 4. The data differ from those so far discussed in being based upon a size-selection from the complete population, whilst the others were derived from a sample of one-tenth of the trees (very slightly biased in favour of the earliest established trees). The average stand of tappable trees per acre for eight experiments was *oP* plots 51.9, *2P* plots 96.0. The nitrogen effect was highly significant except in the two experiments on P.B. 23. The greatest contrast was shown in Experiment No. 1 (see Plate 10), which had only 7 tappable trees per acre on the untreated plot against 90 or more on those receiving both nitrogen and phosphate. There was some slight indication of a negative interaction between nitrogen and potash.

Experiments on mature trees.—Growth-effects in the older buddings are shown in Table 5, based upon a combination of girth-increments over four years. In these more mature trees the growth for this period represents only a small fraction of the girths measured. Although the actual girths have such a high standard error that they fail to prove any results when used directly, the increments are far more sensitive and have shown significant results in each annual interval and in each experiment.

Nitrogen has nearly the same effect as on the immature trees, which means a greater relative effect, since the average growth-rate has been much slower. Although phosphate still has a greater effect than nitrogen, the average phosphate effect (3.4 cm.) is much smaller than that on the immature trees (8.0 cm.). The $N \times P$ interaction is positive and approaches significance.

Putting the two series together, it appears that the greatest effect of phosphate is derived during the period before competition is felt, when the rate of root-development determines the rate of exploitation of fresh territory; whilst nitrogen becomes more important after the onset

TABLE 4. *Analysis of Number of Trees per acre ready for Tapping at Opening, 1939*

Experiment No. Clone . . . Average . . .	1 G. 1 70.0	4 Av. 256 92.0	2 B.D. 5 77.3	8 B.D. 5 85.6	9 P.B. 186 80.4	5 TJ. 1 94.4	10 ¹ P.B. 23 53.8	12 P.B. 23 76.7
<i>Means</i>								
oN . . .	60.6	84.7	69.2	76.8	72.6	88.4	50.0	73.8
iN . . .	72.0	92.7	79.6	88.3	85.6	92.9	56.9	81.7
2N . . .	77.4	99.0	83.3	91.4	83.3	101.9	54.4	74.4
oP . . .	18.4	45.0	47.2	53.7	56.5	76.0	46.3	71.8
iP . . .	86.1	113.5	84.0	97.1	90.0	104.4	56.5	76.0
2P . . .	105.5	117.9	100.7	105.6	95.0	102.8	58.5	82.0
oK . . .	70.2	92.6	81.0	80.5	80.1	96.6	50.1	71.5
iK . . .	68.6	88.8	75.7	88.3	82.2	93.7	52.1	80.5
2K . . .	71.1	95.0	75.3	87.6	79.1	93.1	59.2	77.9
S.E. . . .	± 4.8	± 7.8	± 5.8	± 5.0	± 4.9	± 5.3	± 5.1	± 9.6
<i>Main effects:</i>								
<i>Linear response</i>								
N . . .	+16.8**	+14.3*	+14.1**	+14.6**	+10.7**	+13.5**	+ 4.4	+ 0.5
P . . .	+87.0**	+73.0**	+53.5**	+52.0**	+38.5**	+26.8**	+12.2**	+10.2
K . . .	+ 0.9	+ 2.4	- 5.7	+ 7.1	- 1.0	- 3.5	+ 9.1*	+ 6.3
S.E. . . .	± 3.4	± 5.5	± 4.1	± 3.5	± 3.5	± 3.7	± 3.6	± 6.8
<i>Curvature</i>								
N . . .	- 6.1	- 1.6	- 6.5	- 8.5	-15.3*	+ 4.6	- 9.3	-15.2
P . . .	-48.3**	-64.1**	-20.1*	-35.0**	-28.5**	-29.9**	- 8.2	+ 1.9
K . . .	+ 4.0	+ 9.9	+ 5.0	- 8.5	- 5.2	+ 2.2	+ 5.1	-11.6
S.E. . . .	± 5.8	± 9.6	± 7.1	± 6.1	± 6.1	± 6.5	± 6.2	±11.7
<i>Linear interaction</i>								
N×P . . .	+ 3.3	+ 9.3	+ 0.3	- 0.5	+ 0.4	- 6.9	+ 1.6	-13.8
N×K . . .	- 5.0	-15.3*	- 4.1	+ 3.1	- 5.9	- 0.8	- 8.3	- 2.0
P×K . . .	- 6.8	+ 1.0	+ 1.4	+ 2.0	- 3.3	- 0.3	- 4.0	+ 5.6
S.E. . . .	± 4.1	± 6.8	± 5.0	± 4.3	± 4.3	± 4.6	± 4.4	± 8.3

¹ Experiment No. 10 suffered heavy storm damage in 1936.TABLE 5. *Girth Increments in cm. during 4½ Years' Action of Fertilizers. Average of Five Experiments on Mature Trees. Average Girth-increment 18.21 cm.*

<i>Means</i>				<i>Main effects</i>	
<i>Rate</i>	<i>N</i>	<i>P</i>	<i>K</i>	<i>Linear response</i>	<i>Curvature</i>
0	16.99	16.39	18.31	N +2.17**	-0.89
1	18.51	18.47	18.19	P +3.38**	-0.77
2	19.15	19.77	18.12	K -0.19	+0.05
				S.E. ±0.51	±0.90
S.E. ±0.36					
<i>N×P Table</i>				<i>Linear interactions</i>	
	oN	iN	2N	N×P	+1.28
oP	15.81	16.77	16.60	N×K	-0.34
iP	17.10	18.88	19.43	P×K	+0.25
2P	18.05	19.87	21.39	S.E.	±0.63
S.E. ±0.63					

of competition. In the mature buddings, too, phosphate (to overcome a fundamental soil deficiency) is required in order to bring out the best results from nitrogen. Potash is shown not to be necessary for growth; indeed, the frequency with which small negative values occur suggests careful consideration before employing it.

Bark thickness.—Measurements of bark thickness have been made on one or two occasions, and they show similar effects to those already proved for girth. Table 6 summarizes the results in two experiments with immature trees. The main effect is from phosphate and the difference would seem to express the advances toward maturity of the bark. Measurements on bark-renewal, made on one mature stand which was opened after two years' action of fertilizers, did not show any significant differences due to the treatments on bark up to 7 months' renewal, but there was a positive correlation of high significance ($r = +0.740$ for 27 pairs) between average bark-renewal and girth. This proves that bark-renewal is affected by site differences in the same way as girdling, though the experiment was not sensitive enough to establish the direct effect of manuring on bark-renewal. Site-variation through a block of land in Malaya usually causes much greater differences in yield and growth than the effects which can be achieved by manuring.

TABLE 6. *Effects on Thickness of Virgin Bark after 4 Years' Action*

Expt. No. 4 Clone: Avros 256 Mean: 7.77 mm.				Expt. No. 5 Clone: Tj. 1 Mean: 6.49 mm.			
	N	P	K		N	P	K
0	7.63	7.31	7.83	0	6.47	6.33	6.59
1	7.81	7.88	7.78	1	6.45	6.44	6.43
2	7.85	8.12	7.69	2	6.53	6.69	6.45
S.E. ± 0.070				S.E. ± 0.060			

Main effects			Main effects		
Linear response	Curvature	Linear interactions	Linear response	Curvature	Linear interactions
N +0.22*	-0.13	N×P +0.11	N +0.06	+0.11	N×P +0.03
P +0.81**	-0.32	N×K -0.03	P +0.36**	+0.14	N×K -0.01
K -0.14	-0.04	P×K +0.08	K -0.14	+0.17	P×K +0.03
S.E. ± 0.099	± 0.169	S.E. ± 0.12	S.E. ± 0.085	± 0.148	S.E. ± 0.11

YIELDS

Yield tests are carried out at intervals of one or two months by means of a sampling technique. A proportionate sample of trees is taken and the latex coagulated in the cup. The samples were machined, dried, and weighed individually during the time that full statistics were being collected, but when the sampling technique has been properly proved the bulk weight of dried cup-lump for each plot is sufficient. The sampling error for a given intensity of sampling can be compared with the error of the

experiment to calculate L ,¹ the fractional loss of information due to omission of trees. Each round of sampling provides only an estimate of L so that a number need to be averaged for safe conclusions. The results for budded rubber show that one may count on retaining more than three-quarters of the information when sampling 1 tree in 5, obtaining 30 tree samples from plots of 180 or 200 trees; and this was the standard finally adopted. Table 7 shows the chief data for experimental and sampling errors and percentage loss of information, based upon 10 or 12 rounds for each example cited.

TABLE 7. *Yield Errors, Ranges, and Averages on Various Rounds of Sampling*

Experiment	Clone	Experimental error (per cent. of mean)		Sampling error 30 trees (per cent. of mean)		Average per- centage loss of information L'
		Range	Mean	Range	Mean	
Freshly opened immature group	Various	8.0-22.2	14.3	6.0-10.0	8.43	22.2
No. 6	Av. 50	7.1-25.7	16.8	6.5-9.1	7.46	11.1
7	Av. 49	6.2-19.3	14.7	6.5-9.5	8.37	24.4
11	Av. 50	8.0-25.2	14.4	5.7-10.6	7.70	21.2
13	Av. 49	11.0-23.7	17.5	5.0-10.2	7.30	10.7
14	Av. 50	11.6-29.7	18.7	6.1-12.4	9.25	15.2
Average			16.07	..	8.08	17.5

Yields for the group of experiments on younger trees during their first months of tapping are shown in Table 8. The average of effects on number of trees tappable (Table 4) are shown together with the effects on yield per tree tapped. Since only two rounds of sampling in a phenomenally dry year are available the evidence for yield is rather slight.

The major practical result is, of course, the increased number of tappable trees at opening, but phosphate has also given a small but highly significant increase in yield per tree, which may be due, at least in part, to the better average size of the tapped trees. It can be estimated that the cost of the five applications of phosphate used to produce the results has been recouped in the first three or four months of tapping.

Field data from the 'mature' group of experiments cover the early years of tapping, $5\frac{1}{2}$ years in the oldest experiment and $2\frac{1}{2}$ years in the youngest. Significant responses are shown only on a few occasions, and the positive effects are mainly contributed during periods of dry weather. The average results are very small, the only significant effect

¹ $L = \left(1 - \frac{k}{h}\right) \frac{B}{A}$, where h = number of trees available, k = number sampled, B = sampling mean square, A = experimental error mean square. Before averaging a group of values from identical experiments, they are corrected for bias by the expression: $L' = \left(1 - \frac{2}{n}\right) L$, where n is the number of degrees of freedom for error in the experiment. [See Yates and Zecopany, J. Agric. Sci., 1935, 25, 545.]

after five years of manuring being a small increase for phosphate, as shown in Table 9. Although the effects are so small the conclusion conveyed must be regarded as very important, for it is established beyond doubt that considerable differences in vigour and growth may be

TABLE 8. *Mean of Effects in Eight Experiments on Immature Trees*
Tappable trees per acre: average 78.8

	Means			Main effects		Linear interactions
	N	P	K	Linear response	Curvature	
0	72.1	52.0	77.8	N +11.1**	-7.2*	N×P -0.8
1	81.2	88.5	78.8	P +44.1**	-29.0**	N×K -4.7
2	83.2	96.1	79.8	K +2.0	-0.1	P×K -0.6
	S.E. ±1.10			S.E. ±1.56	±2.70	S.E. ±1.91

Yield per tree per tapping: average 17.9 gm.

	Means			Main effects		Linear interactions
	N	P	K	Linear response	Curvature	
0	17.82	16.71	17.76	N +0.13	-0.07	N×P +0.26
1	17.92	18.25	17.93	P +2.02**	-1.05	N×K -0.36
2	17.95	18.73	18.01	K +0.25	-0.08	P×K +0.36
	S.E. ±0.25			S.E. ±0.35	±0.60	S.E. ±0.42

produced by the manuring of young rubber without much immediate effect upon yield. For instance, the double-nitrogen and phosphate plots have shown a 35.0 per cent. benefit on girth-increment but only

TABLE 9. *Mean Yield Effects as gm. per Tree per Tapping, in Five Experiments in the Fifth and Sixth years of Manuring. Average yield 25.0 gm.*

	Means			Main effects		Linear interactions
	N	P	K	Linear response	Curvature	
0	24.43	23.86	25.07	N +0.70	-1.30	N×P +0.87
1	25.43	25.39	24.83	P +1.91**	-1.16	N×K +0.33
2	25.13	25.77	25.09	K +0.02	+0.50	P×K -0.51
	S.E. ±0.38			S.E. ±0.54	±0.93	S.E. ±0.65

a 12.8 per cent. benefit on yield. There is a very considerable time-lag before differences in growth appear with full effect on the yield, for, first, the bark quality must develop differences (which may only occur on renewed bark), and, secondly, this bark must be reached in the cycle of tapping. It is therefore dangerous to await experimental proof of economic yield-increases on an area before deciding whether manuring should be undertaken, for deterioration will probably have been at work for some years before such proof is afforded by yields. The chain of action is: depleted foliage → slower growth → poorer bark renewal → reduced yield from tapping such renewed bark, and it is not surprising

that yield effects lag a long time behind the initiation of those changes which are ultimately responsible for them. In practice it is important to decide upon manuring measures at the first sign of deterioration in leaf-canopy.

Although interactions are hardly to be expected when main effects are so small, it may be worth while pointing out that no anomalous yield effects have appeared with any particular treatment. The suggestion that unbalanced action is undesirable has received no supporting evidence.

Further tests of the phosphate effect.—Since the first demonstration of the importance of phosphate, a number of further tests have been undertaken, and in no case have they failed to produce marked responses.

TABLE 10. *Comparison of Three Forms of Phosphate, giving equal P_2O_5 at Two Rates, with and without Nitrogen. (6 replicates of 16 treatments)*

Annual girth-increments in cm. for Clone B.D. 5

(a) Averaging with and without N

	No P	Cheribon		Christmas Island		Double superphosphate		S.E.
		1P	2P	1P	2P	1P	2P	
1936 . .	8.19	8.76	8.77	8.73	8.99	9.46	9.68	± 0.222
1937 . .	7.20	8.03	8.33	7.96	8.13	8.38	8.48	± 0.162
1938 . .	4.74	6.21	6.87	5.72	6.54	6.04	6.60	± 0.194
1939 ¹ . .	2.95	3.19	3.35	2.91	2.94	2.66	2.76	..
4 years . .	23.08	26.19	27.32	25.32	26.60	26.54	27.52	..
Difference	+3.11	+4.24	+2.24	+3.52	+3.46	+4.44	..

(b) Averaging single and double dressings of phosphates

	No P		Cheribon		Christmas Island		Double superphosphate		S.E.
	O	N	O	N	O	N	O	N	
1936 . .	8.40	7.98	8.65	8.88	8.53	9.19	9.34	9.79	± 0.222
1937 . .	7.09	7.31	8.43	7.92	7.76	8.33	8.41	8.45	± 0.162
1938 . .	4.76	4.72	6.52	6.56	5.99	6.26	6.28	6.36	± 0.194
1939 ¹ . .	2.97	2.93	3.31	3.23	2.94	2.92	2.82	2.60	..
4 years . .	23.22	22.94	26.91	26.59	25.22	26.70	26.85	27.20	..
Difference	-0.28	..	-0.32	..	+1.48	..	+0.35	..

¹ Tapping started in 1939: this reduced the growth-rate, especially on the most advanced plot.

In a comparison of superphosphate with two forms of raw rock phosphate (Cheribon and Christmas Island) on the basis of equal total P_2O_5 , measurements were taken every two months because an early answer was important for commercial practice. A period of induction after the first application was indicated. Response in stem-growth was delayed for several months, the first reaction presumably being in the roots, with a temporary deflexion of energy which may even slightly depress stem-growth. Superphosphate gave the quickest and best results, but the rock phosphates were not a long way behind, establishing their effects more slowly, as shown in Table 10. Nitrogen had no effect by itself,

but during the first year it counteracted a depressive effect by which the double dressing of phosphate gave less response than the single. Nitrogen also showed a positive interaction with Christmas Island phosphate (1.50 cm. better growth in 3 years with nitrogen than without) but none of consequence with the other forms. The figures for the fourth year are included to show how tapping temporarily upsets the growth figures.

In a replanting on badly eroded hills with stiff white clay, where the former stand of trees had never succeeded well and had been long abandoned, phosphate was found to make all the difference between complete failure and complete success. Neither nitrogen nor potash was of importance at first, but nitrogen assumed more importance after

TABLE 11. *Replanting Experiment*

(a) *Average girths in cm. at four years (General mean 26.3 cm.)*

Means				Main effects		Linear interactions
Rate	N	P	K	Linear responses	Curvature	
0	25.0	21.2	26.4	N +3.2**	N +1.0	N×P +1.1
1	26.0	28.3	26.3	P +8.3**	P -5.8**	N×K +0.7
2	28.1	29.5	26.2	K -0.3	K -0.1	P×K -1.1
S.E. ±0.48				S.E. ±0.68	S.E. ±1.18	S.E. ±0.84

(b) *Percentage of sky covered by leaf¹ (General mean 60.2 per cent.)*

Means				Main effects		Linear interactions
Rate	N	P	K	Linear responses	Curvature	
0	47.7	36.9	60.9	N +23.8**	N -3.8	N×P -7.4
1	61.6	66.7	61.3	P +40.5**	P -19.2	N×K -3.4
2	71.6	77.3	58.8	K -2.2	K -3.0	P×K +0.8
S.E. ±3.7				S.E. ±5.2	S.E. ±9.1	S.E. ±6.4

¹ The data for percentage of sky covered by leaf were obtained from a large number of visual estimates made by means of a reflex camera pointed vertically and set up a number of times in each plot, the ground glass screen being ruled in squares, and each square of the image separately assessed.

two or three years. Accurate measurement of the actual effect was sacrificed by giving a small basal dressing of 1.5 oz. of rock phosphate per tree after 9 months to save the controls from total loss, but as an indication of the position it may be stated that phosphate plots (budded with Tj. 1) reach an average girth of 9.75 in. (at 40 in.) at 3½ years from planting. Plate 11 shows the development at four years in this experiment by means of a representative photograph on each plot taken vertically from the ground between the trees. Girth-figures and numerical estimates of proportion of sky covered by leaf are given in Table 11 for the same plots. Both girths and foliage showed highly significant responses to the double dressing of nitrogen and the single dressing of phosphate, but the further response to the second unit of phosphate was not significant.

Other tests showed basic slag to give the best results in the first year

or so from planting, but this superiority was not maintained. Also, a very small dose of phosphate gave the best results at planting; 0.15 oz. of citric-soluble P_2O_5 per planting-point gave significantly better results than either twice or four times this amount (Curve 1a in Fig. 3). The demands of the tree naturally increase very rapidly after its first year.

The intimate connexion between the magnitude of the phosphate

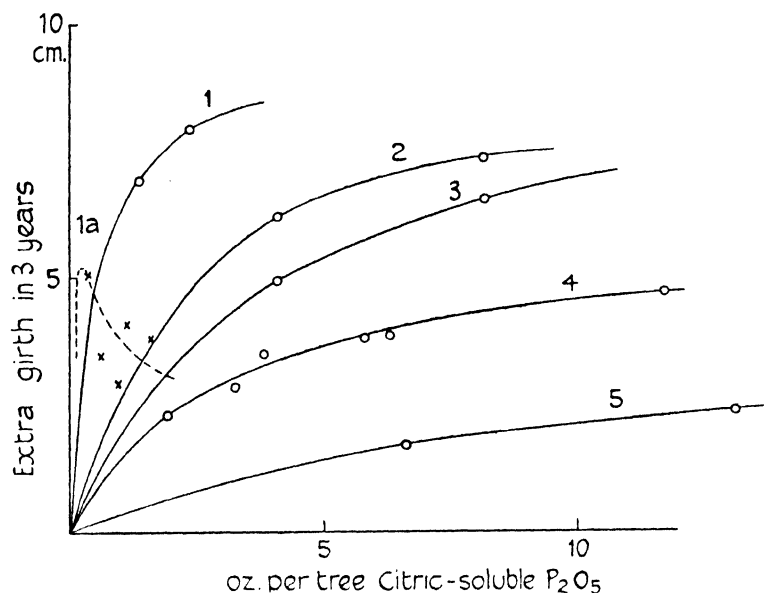


FIG. 3. Phosphate effect for different ages.

1a.	Earliest effects on stock, 18 months, after planting	oP growth 13.0 cm.
1.	Tj. 1, manured from planting, 3 years' effect from budding	18.5 "
2.	Tj. 1, manured 1½ years from budding, effect in first 3 years of manuring	18.3 "
3.	B.D. 5, manured 1½ years from budding, effect in first 3 years of manuring	20.0 "
4.	B.D. 5, manured 3½ years from budding, effect in first 3 years of manuring	20.2 "
5.	Av. 49 and 50, manured at maturity, effect in first 3 years of manuring	13.7 "

response and the age of the trees when treatment is started is well illustrated in Fig. 3, which brings together a number of independent results. In all cases except the dotted line 1a, the result of three years' action with annual manuring is plotted against the total amount of citric-soluble P_2O_5 given, taking into consideration those applications which have had time to contribute their effects. Curve 1 refers to the replanting experiment and shows the response for the first 3 years after budding of a stand of Tj. 1, manured from first planting. This case is not strictly comparable to the others, as the soil is not virgin, and the controls had received a small basic dressing of phosphate at 9 months to save them from complete failure.

Curve 1(a) in dotted line refers to stock growth for 18 months from planting in the same area as 1. Curves 2 and 3 represent two extreme cases in the main group of experiments, Clones Tj. 1 and B.D. 5, respectively, first manured $1\frac{1}{2}$ years after budding. Curve 4 covers the data of Table 10 and refers to the same clone in the same field as curve 3. But manuring started 18 months later than for 3 (in the year of maximum growth) and the response is diminished by nearly 'missing the tide.' Curve 5 represents the average growth of the mature trees manured at 5 or 6 years after budding.

The conclusion clearly is that the major growth-effect from phosphate is to be reaped only during the immature stages. The effect is best initiated at planting with very restrained dosage, but a rapid increase should be made at a year from budding. During the next 2 or 3 years a dosage even greater than the double dressing used in the experiments would seem to be fully economic.

Acknowledgements

Acknowledgements are due to a large number of helpers who have worked on and supervised these experiments. Foremost should be mentioned Mr. E. C. Tommerup, who was responsible for laying out the plots and arranging the first applications, and whose schedules have proved remarkably apt. Much help has been afforded by Mr. T. S. A. Iyer, and in the computational work by Y. M. Kim and C. C. Swee. Our thanks are also due to Dunlop Malayan Estates Ltd. for permission to publish.

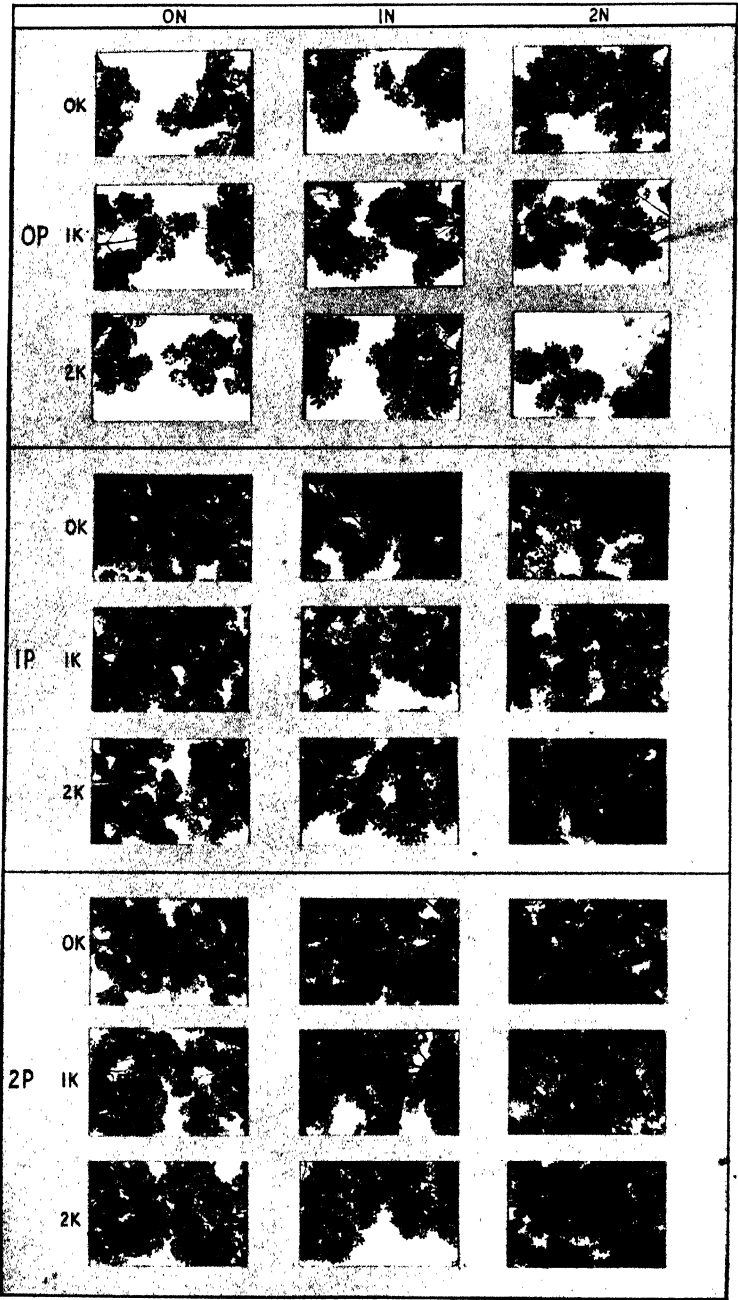
(Received April 7, 1940)



Looking down a double-phosphate plot with no-phosphate plots on either side. Heavy shade overhead and good ground cover: poor shade seen at either side. High proportion of trees in tapping



Looking down a no-phosphate plot with phosphate plots on either side. Poor shade overhead and poor cover: heavy shade to either side. Low proportion of trees in tapping



Representative photos taken from ground in 4-year-old 27-plot Replanting Experiment. All plots had a very small basal dressing of phosphate in the first year

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THE JOINT DISTRIBUTION OF VARIANCE RATIOS BASED ON A COMMON ERROR MEAN SQUARE

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1. INTRODUCTION

FISHER (1929) derived the exact distribution of the ratio of the largest of a set of sums of squares, each based on two degrees of freedom, to the total of the set. The solution to a geometrical problem, given by STEVENS (1939), was later (1940) shown by FISHER to be an equivalent result, and also to give the distribution of the second, third, etc., largest of the set. GARWOOD (1940) has shown that the same mathematical solution applies to yet a third problem. COCHRAN (1941) has discussed the generalization of the problem to sums of squares based on any number of degrees of freedom.

It is the purpose of the present note to show the solution to the analogous problem of the distribution of the ratio of the largest of a number of mean squares, all based on the same number of degrees of freedom, to an independent 'error' mean square. Methods of obtaining approximate significance levels for the ratio have been discussed by HARTLEY (1938), but, so far as is known, the exact algebraic form of the probability distribution has not previously been given.

2. THE FORM OF THE DISTRIBUTION

If v, v_1, v_2, \dots, v_k are independent sums of squares of deviations of observations in a normal population, the first based on $2n$ and the remainder each on $2m$ degrees of freedom (m, n being positive integers or half integers), the distribution of v is of the form

$$df = \left(\frac{1}{2\sigma^2} \right)^n \frac{1}{\Gamma(n)} v^{n-1} e^{-v/2\sigma^2} dv, \quad (1)$$

the distributions of the v_i being similar, where σ^2 is the variance of the normal population. The joint distribution of the ratios

$$\phi_i = \frac{v_i}{v} \quad (2)$$

may be shown to be

$$df = \frac{\Gamma(n+km)}{\Gamma(n)\{\Gamma(m)\}^k} \prod_{i=1}^k \{\phi_i^{m-1}\} \left(1 + \sum_{i=1}^k \phi_i \right)^{-(n+km)} \prod_{i=1}^k (d\phi_i), \quad (3)$$

which may be described as the 'Studentized' form of the joint distribution of v_1, \dots, v_k , the latter distribution having been modified so as to give the distribution of statistics involving only quantities calculable from a sample and independent of population parameters.

On the analogy of the case $k = 1$, which leads to the well-known variance ratio and z -distributions, it seems of interest to make the transformation

$$\phi_i = \frac{m}{n} e^{2z_i}. \quad (4)$$

The resulting distribution is

$$df = 2^k \left(\frac{m}{n}\right)^{mk} \frac{\Gamma(n+mk)}{\Gamma(n)\{\Gamma(m)\}^k} \frac{e^{2m \sum_i z_i}}{\left(1 + \frac{m}{n} \sum_i e^{2z_i}\right)^{n+km}} \prod_i (dz_i); \quad (5)$$

and the characteristic function of this distribution is

$$M(t_i, \dots, t_k) = \left(\frac{n}{m}\right)^{\frac{1}{2} \sum_i t_i} \frac{\Gamma(n - \frac{1}{2} \sum_i t_i) \prod_i \{\Gamma(m + \frac{1}{2} t_i)\}}{\Gamma(n)\{\Gamma(m)\}^k}. \quad (6)$$

3. THE PROBABILITY INTEGRAL

It will be convenient to make use of the operator

$$D = \frac{\partial}{\partial \lambda}, \quad (7)$$

and to define fractional powers of D by the relation

$$D^t(\mu + \lambda)^{-\nu} = (-1)^t \frac{\Gamma(p+t)}{\Gamma(p)} (\mu + \lambda)^{-(\nu+t)} \quad (8)$$

for all positive p and t , μ being a quantity independent of λ . Then it is well known that

$$e^D f(\lambda) = f(\lambda + 1). \quad (9)$$

The operators L , M may be defined by

$$L = 1 - M = \frac{1}{\Gamma(m)} \int_0^{-aD} u^{m-1} e^{-u} du = \Gamma_{-aD}(m) \quad (10)$$

in the usual incomplete Γ -function notation. If m is an integer, L may be expanded in a terminating series and is

$$L = e^{aD} \left(1 - aD + \frac{1}{2!} a^2 D^2 - \dots + \frac{(-1)^{m-1}}{(m-1)!} a^{m-1} D^{m-1} \right), \dots \quad (11)$$

but if m is not an integer, it is preferable to make use of the infinite series

$$M = e^{aD} \left\{ \frac{1}{m!} (-aD)^m + \frac{1}{(m+1)!} (-aD)^{m+1} + \dots \right\}. \quad (12)$$

By means of successive integration by parts it may be shown that

$$\int_0^a \frac{\phi^{m-1}}{(\mu + \lambda + \phi)^{p+m}} d\phi = \frac{\Gamma(p)\Gamma(m)}{\Gamma(p+m)} M \left\{ \frac{1}{(\mu + \lambda)^\mu} \right\} \quad (13)$$

for all positive p and m and any μ independent of λ . The series on the right-hand side of (13) will converge provided only that $\mu + \lambda + a > 1$.

Now, from (3), the probability that the k values of ϕ should all be less than a is

$$P\{\phi_i < a; i = 1, 2, \dots, k\} = \frac{\Gamma(n+km)}{\Gamma(n)\{\Gamma(m)\}^k} \int_0^a \dots \int_0^a \frac{(\phi_1 \phi_2 \dots \phi_k)^{m-1}}{(1 + \phi_1 + \phi_2 + \dots + \phi_k)^{n+km}} d\phi_1 d\phi_2 \dots d\phi_k,$$

and successive applications of the equation (13) reduces this and shows the probability to be equal to

$$\frac{M^k}{(1 + \lambda)^n}, \quad (14)$$

where, after differentiation, λ is to be put equal to zero. A more general result, which is obtained by the same method, is the probability that exactly r of the k ratios of sum of squares are less than a ; this probability is equal to

$${}^kC_r L^{k-r} M^r \frac{1}{(1+\lambda)^n}, \quad (15)$$

where again λ is put equal to zero after differentiation.

4. TABULATION OF SIGNIFICANCE LEVELS

It would be possible to tabulate significance levels for the largest of k variance ratios in the same form as the existing tables for a single variance ratio (Fisher & Yates, 1938, Table V) by finding for sets of values of k, m, n the value of na/m which gives to the probability (14) the values 0.95, 0.99, etc. Unfortunately the complexity of the algebraic expression of (14) in terms of a increases rapidly as the degrees of freedom increase. The simplest cases are those which have m integral. Thus with $m = 1$ there is obtained the probability that all of k variance ratios with $(2, 2n)$ degrees of freedom are less than na ; this is

$$P = 1 - \frac{{}^kC_1}{(1+a)^n} + \frac{{}^kC_2}{(1+2a)^n} - \dots + \frac{(-1)^k}{(1+ka)^n}. \quad (16)$$

The corresponding probability for $(4, 2n)$ degrees of freedom, given by $m = 4$, is the probability that all the k variance ratios are less than $na/2$,

$$\begin{aligned} P = 1 - \frac{{}^kC_1}{(1+a)^n} & \left[1 + \frac{an}{(1+a)} \right] \\ & + \frac{{}^kC_2}{(1+2a)^n} \left[1 + \frac{2an}{(1+2a)} + \frac{a^2n(n+1)}{(1+2a)^2} \right] \\ & \dots \dots \dots \\ & + \frac{(-1)^k}{(1+ka)^n} \left[1 + \frac{{}^kC_1 an}{(1+ka)} + \frac{{}^kC_2 a^2 n(n+1)}{(1+ka)^2} + \dots + \frac{a^k n(n+1) \dots (n+k-1)}{(1+ka)^k} \right]. \end{aligned} \quad (17)$$

These expressions may be used to give the significance levels shown in Table 1 below. The first column is taken from Table V of Fisher & Yates (1938), and the second and third are obtained by inversion of (16) and (17) respectively.

Approximations to the significance levels may, however, be obtained by assuming the variance ratios to be independent, as has been suggested by Hartley (1938). This approximation may be expressed symbolically as

$$P\{\phi_i < a; i = 1, 2, \dots, k\} \simeq \prod_{i=1}^k P\{\phi_i < a\} = [P\{\phi < a\}]^k, \quad (18)$$

since the probabilities for the separate ratios are equal. Theoretical discussions of the accuracy of this approximation have so far been unsuccessful. Calculation shows that for the case of $m = 1$ it is reasonably satisfactory if n is not too small. The agreement between approximate and exact values will presumably improve as m increases, so that the chief uncertainty appertains to the case $m = \frac{1}{2}$.

On general grounds it may be argued that the upper significance levels thus approximately obtained will exceed their true values, and thus the significance of results will be under- rather than over-estimated. The truth of this is obvious in the case $m = 1$, for which the approximate formula gives as an approximation to (16)

$$P = \left\{ 1 - \frac{1}{(1+a)^n} \right\}^k. \quad (19)$$

Table 1. 5 % significance levels of the largest of k variance ratios with $(2, 2n)$ degrees of freedom

$\begin{smallmatrix} k \\ \backslash \\ 2n \end{smallmatrix}$	1	2	3
1	199.5	333.7	423.3
2	19.00	28.83	35.43
3	9.55	13.74	16.50
4	6.94	9.67	11.45
5	5.79	7.88	9.24
6	5.14	6.90	8.03
7	4.74	6.28	7.27
8	4.46	5.86	6.75
9	4.26	5.55	6.38
10	4.10	5.32	6.09
20	3.49	4.41	4.98
∞	2.99	3.68	4.08

Table 2. Comparison of true and approximate 5 % significance levels of the largest of k variance ratios with $(2, 2n)$ degrees of freedom

$2n$	$k = 2$			$k = 3$		
	Significance level		Probability of approx. value	Significance level		Probability of approx. value
	True	Approximate		True	Approximate	
1	333.7	779.4	0.0327	423.3	1738	0.0247
2	28.83	38.49	0.0378	35.43	57.99	0.0309
3	13.74	15.90	0.0411	16.50	21.23	0.0354
4	9.67	10.57	0.0432	11.45	13.36	0.0386
5	7.88	8.38	0.0446	9.24	10.27	0.0408
6	6.90	7.22	0.0456	8.03	8.68	0.0424
7	6.28	6.51	0.0463	7.27	7.72	0.0435
8	5.86	6.03	0.0468	6.75	7.08	0.0444
9	5.55	5.69	0.0472	6.38	6.64	0.0451
10	5.32	5.43	0.0475	6.09	6.30	0.0457
20	4.41	4.44	0.0489	4.98	5.03	0.0481
∞	3.68	3.68	0.0500	4.08	4.08	0.0500

The discrepancy will increase with k , but, in practice, such increase would probably be compensated by an increase in the error degrees of freedom, $2n$. In all cases the approximation becomes exact as $n \rightarrow \infty$, when the limiting χ^2 distributions are obtained.

In Table 2 the true and approximate 5 % significance levels are compared for $m = 1$ and for $k = 2, 3$, the actual probability associated with the approximate figures being also shown.

For practical purposes the approximation is sufficiently accurate in either case when the number of degrees of freedom for error exceeds 10. The corresponding table for the 1% level shows the same order of accuracy, but larger values of k would need an increase in the error degrees of freedom for this accuracy to be reached.

5. FURTHER RESULTS

The probability given in (15) may be further generalized to the case of k sums of squares with $2m_1, 2m_2, \dots, 2m_k$ degrees of freedom, leading to a test of significance on the largest of the ratios of the sums of squares in this instance also. But there seems little likelihood of this problem having any practical interest and it will not be further discussed here.

A test of the smallest of k variance ratios, each with $(2m, 2n)$ degrees of freedom, may be derived from the general formula (15). The probability that all the k ratios of sums of squares are greater than a is

$$P\{\phi_i < a; i = 1, 2, \dots, k\} = L^k \frac{1}{(1 + \lambda)^n}. \quad (20)$$

The convergence of the series by which significance levels for the smallest variance ratio are obtained is much more rapid than for the largest ratio. Also, as Hartley has demonstrated, the approximation given by an assumption of independence as in (18) is very close even for small numbers of degrees of freedom. Indeed for $m = 1$ the two expressions are identical, the result being

$$P = (1 + ka)^{-n}. \quad (21)$$

6. SUMMARY

The distribution has been obtained of the largest of a number of variance ratios, such as occur in an analysis of variance when a number of independent mean squares with the same number of degrees of freedom are all compared with the same error mean square. It is found that the computation of exact significance levels is a lengthy process on account of slowness of convergence, but the approximation resulting from an assumption of complete independence of the ratios is satisfactory providing that the error degrees of freedom are sufficiently numerous.

The solution of the less interesting problem of obtaining significance levels for the least of the variance ratios is arithmetically much simpler, and the similar approximation very satisfactory.

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MODERN EXPERIMENTAL DESIGN AND ITS FUNCTION IN PLANT SELECTION¹

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1. *Advantage of including a large number of varieties in field trials.*—Plant-breeding trials differ from most other types of agronomic experiment in the large number of varieties or lines that are available for comparison.

Obviously the greater the number of varieties included at any stage of the selection programme the less is the chance of some particularly good variety being rejected without trial. On the other hand, the comparisons between the individual varieties will be less accurate, owing to the smaller number of plots that can be devoted to each variety, and owing to the increased error per plot as the number of varieties is increased.

Neglecting the latter factor entirely, and assuming that the total number of plots is fixed, the average genetic advance due to the selection of the apparently best variety, instead of a random variety, will be

$$\frac{G}{G + \lambda n} \bar{x}_n,$$

where n is the number of varieties,

G is the genetic variance (distribution assumed normal),

λn is the experimental error variance, λ being independent of n ,

\bar{x}_n is the mean value of the greatest deviate of a sample of n from a normal population with unit standard deviation. (Tabulated in [3].)

With $\lambda = \frac{1}{10}G$ the optimum number of varieties will be 13, in which case the genetic variance will be somewhat less than the experimental error variance. With $\lambda = \frac{1}{100}G$ the optimum number will be somewhat greater than 50, and the genetic variance will be about twice the experimental error variance. In terms of the ordinary analysis of variance the variance ratios between varieties and error will average about 1.8 and about 3 respectively. The former is less than the value required to give significance at the 5 per cent. point.

These simple considerations serve to emphasize the value of testing a large number of varieties with moderate accuracy instead of only a few with very high accuracy. In any series of trials involving only a few varieties which give varietal differences that are large compared with their standard errors the question should always be asked: would not the experiments have been improved if the same experimental resources had been devoted to the comparison of a larger number of varieties?

When a large number of varieties has to be compared Latin squares are impossible, and randomized blocks begin to lose their efficiency owing to the large number of plots per block. In the past, attempts have been made to overcome this difficulty by the use of controls. It has now been shown [1], however, that except when the available amount of seed

¹ Read at the Seventh International Genetical Congress, Edinburgh, August 1939.

of the new varieties is a limiting factor, the use of controls is unlikely to be as efficient as ordinary randomized blocks, however the controls are arranged, owing to the large amount of ground that must be devoted to the control varieties.

The designs described here may be looked on as devices for making the new varieties act as controls for one another. In essence they are merely randomized blocks with certain additional restrictions which enable the fertility differences to be more fully eliminated. They do not introduce any additional complications in the field, and require only a moderate increase in the amount of statistical calculation over that required for ordinary randomized blocks.

These designs are of interest not only to the plant breeder, but in many other branches of biological experimentation. In animal work, for instance, they can be used to eliminate the effects of litters when these contain fewer animals than there are treatments, and at the same time the litter differences can be freed from treatment effects, and hence evaluated exactly. This alone opens a wide field for combined experiments on breeding and treatments.

2. *Classification of Designs.*—There are several variants of these designs, most of which can be included under the following heads:

1. Quasi-factorial arrangements.
2. Balanced arrangements in incomplete blocks.
3. Lattice squares.

Quasi-factorial and incomplete block arrangements possess the common feature that the area under experiment is divided into blocks, each of which contains only a few of the varieties. The arrangement is so made that it is possible to eliminate, either partially or completely, as desired, the yield differences resulting from differences in blocks. In the case of lattice squares each replication is arranged in a square, and differences between rows and columns of each square can be eliminated, as in a Latin square.

3. *The simple lattice.*—The simplest type of arrangement is the two-dimensional quasi-factorial arrangement, or simple 'lattice', and this will serve to illustrate the principles involved. It is best applied to a number of varieties which is a perfect square.

Suppose, for example, we have 49 varieties, to be arranged in 6 replications. We first construct the 'lattice' shown in Fig. 1, and assign the 49 varieties to the intersections at random. Next, we divide up the 294 plots into 6 large blocks, each of which will contain a replication, and subdivide each large block into 7 small blocks of 7 plots each.

There will be two different types of replication. In the first type each of the small blocks will be made up solely of varieties falling in the same column of the lattice, i.e. having the second of the two index numbers the same. The columns will be assigned to the blocks at random and the varieties within the column will be assigned to the plots of the block at random. Thus the first block might contain column 5, the varieties being arranged in the order:

35, 55, 15, 75, 65, 25, 45.

Fig. 2 shows a replication of this type.

The other type of replication is exactly similar, except that the small blocks are made up of varieties falling in the same row of the lattice. The two types must be equally represented.

It will thus be seen that as far as arrangement on the ground is concerned there is little difference from ordinary randomized blocks: there are merely a few additional restrictions in the arrangement. It is there-

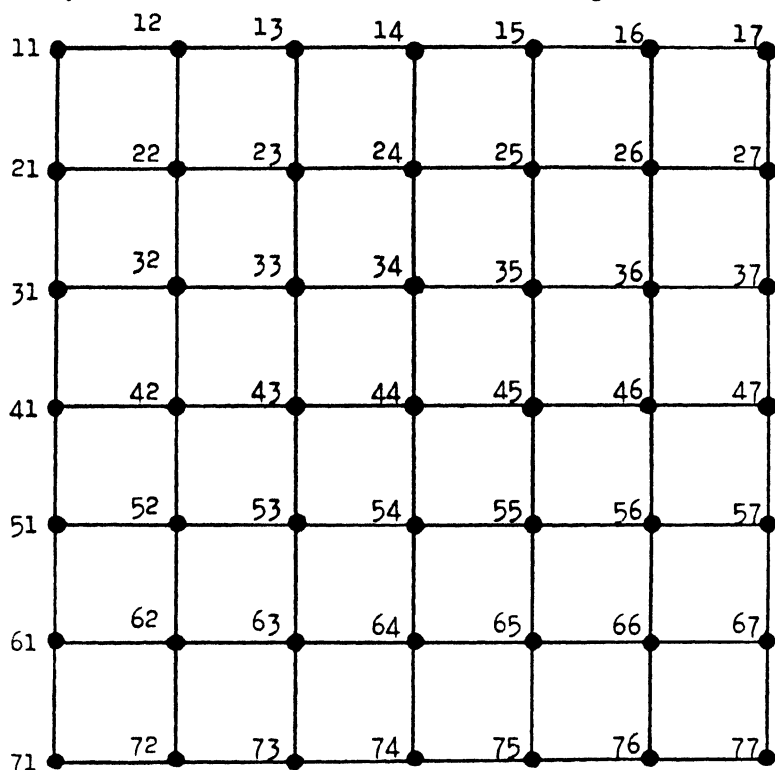


FIG. 1. 7×7 Simple lattice.

fore of interest to note at this stage that the computations may be carried out as if the experiment were one in ordinary randomized blocks, using the ordinary varietal means as estimates of the varietal differences.

If the small blocks are found to differ greatly among themselves, however, these differences may be eliminated entirely from the varietal comparisons in virtue of the special features of the arrangement. To do this we set out the total yields of each variety in each type of replication in the manner of Table 1, taking the marginal totals. Then the fully adjusted yield of variety 11 will be

$$\frac{1}{8}(a - \frac{1}{7}A + \frac{1}{7}A' + b - \frac{1}{7}B + \frac{1}{7}B').$$

A little consideration will show that this expression is independent of block differences and of varietal differences other than that of variety 11. Thus an increase δ in the yields of all the plots of one of the column blocks containing the varieties $x1$ will increase a by an amount δ and A

35	22	11	27
55	12	31	77
15	52	51	47
75	42	41	37
65	72	71	67
25	32	21	17
45	62	61	57
26	23	34	
66	33	54	
16	13	74	
46	63	14	
56	53	64	
76	43	44	
36	73	24	

FIG. 2. Field layout of one replication of a 7×7 simple lattice.

by an amount 7δ : hence $a - \frac{1}{7}A$ remains unchanged. Similarly, an increase in the yield of variety 21 will increase both A and B' by 3 times the amount of the increase: hence $-\frac{1}{7}A + \frac{1}{7}B'$ remains unchanged.

TABLE I. *Method of Eliminating Varietal Differences*

Replications with column blocks								Replications with row blocks											
		Column of lattice						Total			Column of lattice						Total		
		1	2	3	4	5	6				7	1	2	3	4	5		6	7
Row of lattice	1	<i>a</i>	-	-	-	-	-	-	<i>A'</i>	Row of lattice	1	<i>b</i>	-	-	-	-	-	-	<i>B</i>
	2	-	-	-	-	-	-	-	-		2	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-		3	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-		4	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-		5	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-		6	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	-		7	-	-	-	-	-	-	-	-
Total		<i>A</i>	-	-	-	-	-	-	-	Total		<i>B'</i>	-	-	-	-	-	-	-

A slight modification of the analysis of variance is also necessary in order to evaluate the errors of the fully adjusted yields. The details are given in [1].

By this method of computation we have rejected entirely the information provided by the comparisons between plots falling in the different small blocks, whereas by analysing the experiment as if it were one in ordinary randomized blocks we have given equal weight to all comparisons.

In general the most accurate estimates will lie somewhere between the two sets of estimates thus obtained, since the comparisons between plots falling in different blocks will be less accurate than those between plots falling in the same block, but will still provide some information.

The best estimates are therefore given by a weighted mean of these two sets. These may be called the *partially adjusted* yields. To make this procedure possible, the experiment must be sufficiently large to furnish an estimate of the relative accuracy of inter- and intra-block comparisons, a knowledge of which is necessary to determine the proper weighting.

Although the procedure of weighting sounds somewhat elaborate, it does not actually require appreciably more computation than does the calculation of the fully adjusted yields, for a somewhat different method of computation has been devised which gives the partially adjusted yields directly without any calculation of the fully adjusted yields.

The origin of the term 'quasi-factorial' may here be noted. If we took all combinations of two factors each at 7 levels and confounded the main effects of one factor in one set of replications, and the main effects of the other in the other set, we should arrive at an arrangement exactly similar to that described.

4. *Other lattice designs utilizing randomized blocks.*—There are a number of variants and elaborations of the simple lattice. One is the three-dimensional lattice, in which the number of varieties should form a perfect cube. The varieties are then allotted to the intersections of a cubic lattice, the three sets of lines of the lattice give three sets of groupings into blocks. The cubic lattice requires rather more elaborate computations than the two-dimensional lattice, but is useful when the number of varieties is very large. A preliminary account of an experiment on 729 ($= 9^3$) strains of *Ponderosa* pine seedlings arranged in blocks of 9 plots is given in [2].

Returning to the two-dimensional lattice, we may introduce a third set of lines in the lattice bearing a Latin-square relationship to the other two sets, and use these to give a third type of replication. We then obtain a lattice diagram of the type shown in Fig. 3. This is called a triple lattice; as might be expected it somewhat improves the accuracy of the design, but at the expense of greater elaboration in the computations.

The process may be continued until, with a 7×7 lattice, 8 sets of lines, giving 8 different types of replication, are included, all of which have the property that each line of any one set intersects all the lines of each other set once and once only. We then arrive at a *balanced* lattice.

In balanced designs comparisons between all pairs of varieties are of equal accuracy and the computations are particularly simple. Consequently balanced designs should always be used when the number of replications is sufficient and when they are combinatorially possible. The combinatorial problem is equivalent to finding a complete set of orthogonal squares. This is known to be possible for primes and powers of primes and has been shown to be impossible for squares of side 6. Complete sets of orthogonal squares of sides 3, 4, 5, 7, 8, and 9 are given in [3]; the key combinations from which sets of squares of sides 16, 25, 27, 32, 49, 64, 81, and 125 can be formed are given in [4].

5. *Balanced Incomplete Block Designs*.—Balanced lattices are a special type of the more general balanced incomplete block designs. In balanced incomplete blocks the number of varieties is not necessarily an exact multiple of the number of plots in a block. The necessary condition for

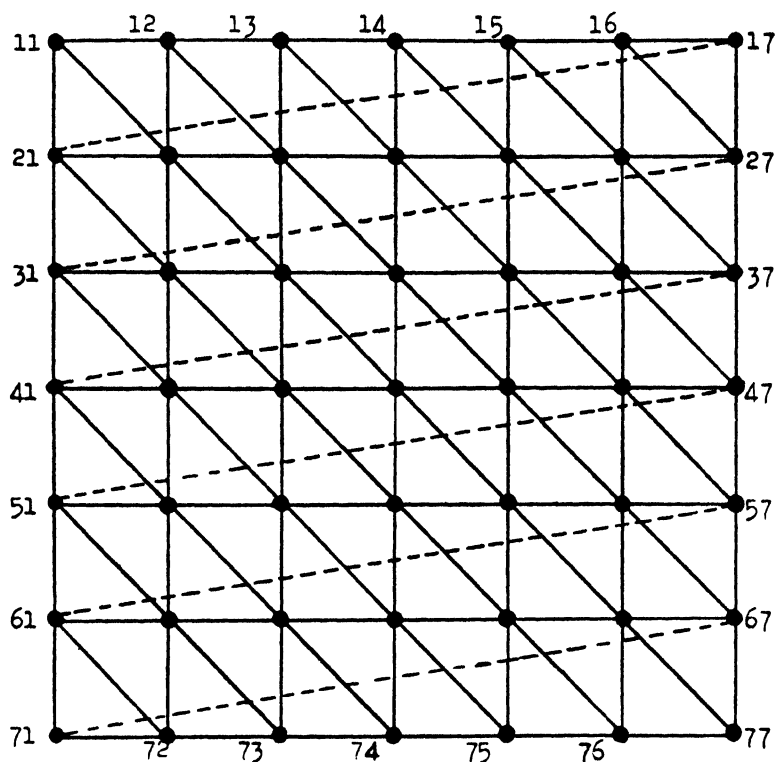


FIG. 3. 7×7 Triple lattice.

balance is that every pair of varieties occurs together the same number of times in a block. Thus, to take a simple example, 7 varieties may be arranged in 7 blocks of 3 plots in the following manner:

<i>abc</i>	<i>bdf</i>	<i>cef</i>
<i>ade</i>	<i>beg</i>	
<i>afg</i>	<i>cdg</i>	

The combinatorial problems to which these designs give rise are quite complex. An index of possible designs with the known solutions is given in [3].

These balanced designs are of some interest in co-operative varietal trials on commercial farms, where each farm can undertake only a small experiment, but where a large number of farms are willing to co-operate. Thus 13 varieties may be compared in balanced sets of 4 at 13 farms, each farm testing one set of 4 varieties. At each farm the comparison might be made by means of a 4×4 Latin square. Such experiments, if repeated in

different districts and on different soil types, should lead to far more rapid and certain determination of the most suitable varieties than many of the methods at present adopted.

6. *Lattice squares*.—The balanced lattices give rise to another type of design of very considerable importance in agriculture, namely lattice squares. The 7×7 balanced lattice already described gives eight sets of lines in all, so that if on the ground we set out four 7×7 squares of plots, we may allocate the varieties to these squares in such a manner that the varieties that compose the rows of the four squares correspond to four of the sets of lines, and the varieties that compose the columns correspond to the other four sets of lines. Thus the blocks of the 8 replications of the ordinary balanced lattice are replaced by the rows and columns of the four lattice squares. Every pair of varieties will occur together in one and only one row or column. Row and column differences may then be eliminated entirely, as in an ordinary Latin square. As before, the information contained in the row and column comparisons may also be utilized. If 8 replications are employed, the rows of one set should correspond to the columns of the other, for the row and column comparisons will then be equally represented in all varietal comparisons.

7. *General remarks*.—Nothing has yet been said on the actual gain in accuracy that may be expected from the use of these designs. This gain is, of course, very variable, and may range from zero to 100 or more per cent. The important point to realize is that the designs can never be less accurate than ordinary randomized blocks, and that when no appreciable gain results they can be treated as ordinary randomized blocks. Thus nothing can be lost by adopting them, and in many cases substantial gains in accuracy will be obtained.

An important development in quasi-factorial and incomplete block designs, which has at present been little investigated, is the introduction of subsidiary treatments such as fertilizers, seeding rates, &c., which can be applied to the separate blocks. In plant-selection work such variation in environment is of the utmost importance if a proper relation between environment and genetic type is to be evolved.

It is sometimes thought that the modern designs involving a large number of small plots will not provide material for quality tests, which are so important in most plant-selection work. It should perhaps be emphasized that the bulking of all the plots of a single variety for quality tests must give as good or better results as a single plot of an equivalent area sown under that variety.

The description here given of the various types of design is intended only as an outline, which will serve to give some idea of the types of design available, the occasions on which they can be useful, and the methods of laying them out. In particular it is hoped that enough has been said to show that the designs are very flexible and can be adapted to a great variety of practical conditions.

No attempt has been made in this paper to describe the details of the methods of computation. Table 2 gives the references to publications in which descriptions of the appropriate methods will be found.

TABLE 2. *Literature References to Methods of Computation*

Type of design	Description of design	Description of method of computation	
		Fully adjusted yields	Partially adjusted yields
Two-dimensional	Simple lattice . . .	[1], [5], [8]	(a)
	Triple lattice . . .	[1], [8]	(a)
	Balanced lattice . . .	[5], [6], [8]	(b)
Three-dimensional (cubic) lattice . . .	[1], [5], [8]	[1], [5]	[9]
Balanced incomplete blocks . . .	[3], [5], [6], [8]	[3], [5], [6]	(b)
Lattice squares . . .	[7], [8]	[7]	[10]

(a) Description to be published shortly by members of the Statistical Department of Iowa State College, Ames, Iowa.

(b) Description to be published shortly in England.

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LATTICE SQUARES

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1. INTRODUCTION

THIS is one of a series of papers describing new methods of analysing quasi-factorial and incomplete block designs. These modified methods of computation have as their object the recovery of the information contained in the inter-block or inter-row and inter-column comparisons. The methods applicable to three-dimensional lattices have been described by Yates (1939), where a full discussion of the principles involved will be found.

The present paper deals with the type of design known as quasi-Latin, or lattice, squares. This type of design was first described by Yates (1937). In essence it consists of a set of randomized blocks, in which the plots of each block form a square pattern on the ground, certain restrictions being imposed on the randomization within the blocks. These restrictions enable the differences between the rows and columns of each block to be eliminated, either partially or entirely, from the varietal comparisons.

Any lattice square design must contain a number of varieties (or treatments) which is a perfect square, and a design with p^2 varieties requires either $\frac{1}{2}(p+1)$ or $p+1$ replications. With $\frac{1}{2}(p+1)$ replications (which are only possible if p is odd), the design is balanced as far as intra-row and column comparisons are concerned, but the inter-row comparisons only give information on one-half the varietal degrees of freedom, and the inter-column comparisons on the other half. Thus, if inter-row and inter-column information is utilized, and the row and column comparisons are of differing accuracy, as will frequently occur in practice, there will be some difference in accuracy in the comparisons of the adjusted yields, though this difference in accuracy is never likely to be very large.

With $p+1$ replications, on the other hand, information is obtained on every varietal degree of freedom from both row and column comparisons, and consequently all varietal comparisons will be of equal accuracy, even if the row and column comparisons are of differing accuracy.

Table I. *Set of lattice squares for 25 varieties,
before randomization of rows and columns*

Square I					Square II					Square III				
1	2	3	4	5	1	13	25	7	19	1	15	24	8	17
6	7	8	9	10	20	2	14	21	8	18	2	11	25	9
11	12	13	14	15	9	16	3	15	22	10	19	3	12	21
16	17	18	19	20	23	10	17	4	11	22	6	20	4	13
21	22	23	24	25	12	24	6	18	5	14	23	7	16	5

Table I shows a design for 25 varieties with three replications. The structure of the design is such that every variety occurs once and once only with every other variety either in the same row or the same column of one of the squares. The groups of varieties which form the rows and columns of the three squares are in fact the groups given by the rows and columns and the four orthogonal squares of a 5×5 set of orthogonal squares. As far as is known such sets of orthogonal squares only exist when p is a prime or a power of a prime.¹ Thus lattice squares cannot be used for 36, 100 or 144 varieties.

Before laying out any trial on the ground the numbers must be assigned to the varieties (or treatments) at random, the rows of each square must be interchanged in a random order, and the columns must also be similarly interchanged. With $p+1$ replications the second $\frac{1}{2}(p+1)$ replications are obtained from the first $\frac{1}{2}(p+1)$ replications by turning each square through a right angle so as to interchange rows and columns, and then re-randomizing rows and columns as above.

In the original paper only the complete elimination of row and column differences was described. If, however, the actual variation of rows and columns is small, this results in loss of information. In the limiting case when all three sets of comparisons are of equal accuracy, this loss amounts to $1-E$, where E , the efficiency factor, equals $(p-1)/(p+1)$. The most accurate results can be obtained by determining the actual accuracy of the row and column comparisons relative to the intra-row and column comparisons, and weighting the three sets of comparisons according to their relative accuracy.

It is the object of this paper to describe the computations which are necessary to effect this expeditiously.

¹ Complete sets of orthogonal squares of sides 3, 4, 5, 7, 8 and 9 have been given by Fisher & Yates (1938). Methods of generating sets of larger squares have been given by Stevens (1939).

2. CASE OF $\frac{1}{2}(p+1)$ REPLICATIONS(a) *Subdivision of varietal comparisons into sets of orthogonal degrees of freedom*

If an orthogonal set of squares of side p exists, then from any p^2 quantities $p+1$ sets of totals can be formed corresponding to the groupings given by the $p-1$ orthogonal squares and the rows and columns of the basic square. Each set will contain p totals, each of p quantities.

If the quantities represent the yields of the p^2 varieties, then corresponding to any variety v there will be $p+1$ totals (one from each set) containing this variety. These totals may be denoted by $P_{1v}, P_{1'v}, P_{2v}, P_{2'v}, \dots$. If G is the total of all the yields and y_v is the yield of variety v , then we have the identity $py_v = P_{1v} + P_{1'v} + P_{2v} + P_{2'v} + \dots - G$. Consequently instead of estimating the varietal differences directly, we may estimate the differences within each set of totals.

(b) *Estimation of varietal differences*

Now consider a set of $\frac{1}{2}(p+1)$ lattice squares. Let the row total of the row containing varieties v, v', v'', \dots in the first square be denoted by ${}_IP_{1v}$, and let the totals of these same varieties in the second, third, etc., squares be ${}_{II}P_{1v}, {}_{III}P_{1v}$, etc. Similarly let the column total of the column containing variety v in square I be denoted by ${}_IP_{1'v}$, etc.

From the structure of the squares the comparisons represented by $P_{1'v}$, which are confounded with the rows of square I, are completely unconfounded with both rows and columns of the remaining squares. Therefore if w_r is the weight assignable to row comparisons and w_i the weight assignable to intra-row and column comparisons, the most accurate estimate ${}_wP_{1v}$ of P_{1v} (in units of the total yield of $\frac{1}{2}(p+1)$ plots) is given by

$$\begin{aligned} {}_wP_{1v} &= \frac{1}{2}(p+1) \{w_r {}_IP_{1v} + w_i ({}_{II}P_{1v} + {}_{III}P_{1v} + \dots)\} / (w_r + \frac{1}{2}(p-1) w_i) \\ &= {}_sP_{1v} + \frac{w_i - w_r}{w_r + \frac{1}{2}(p-1) w_i} ({}_sP_{1v} - \frac{1}{2}(p+1) {}_IP_{1v}), \end{aligned}$$

where ${}_sP_{1v} = {}_IP_{1v} + {}_{II}P_{1v} + {}_{III}P_{1v} + \dots$, ${}_sP_{1v}$ being derivable directly from the ordinary table of varietal totals.

Consequently in the case of a square of side p we have the general expressions

$${}_wP_{1v} = {}_sP_{1v} + \lambda L_{1v}$$

for sets confounded with rows, and

$${}_wP_{1'v} = {}_sP_{1'v} + \mu M_{1'v}$$

for sets confounded with columns, where

$$L_{1v} = {}_sP_{1v} - \frac{1}{2}(p+1) {}_IP_{1v}, \quad M_{1'v} = {}_sP_{1'v} - \frac{1}{2}(p+1) {}_IP_{1'v},$$

$$\lambda = \frac{w_i - w_r}{w_r + \frac{1}{2}(p-1)w_i}, \quad \mu = \frac{w_i - w_c}{w_c + \frac{1}{2}(p-1)w_i}.$$

The adjusted total yield of variety v is therefore obtained from the unadjusted total yield by adding quantities

$$\delta_{1v} + \delta_{2v} + \dots + \epsilon_{1'v} + \epsilon_{2'v} + \dots,$$

where $\delta_{1v} = \lambda L_{1v}/p$ and $\epsilon_{1'v} = \mu M_{1'v}/p$, etc.

(c) *The analysis of variance*

Rows and columns are not orthogonal with varieties, and this must be taken into account when calculating the sums of squares in the analysis of variance.

As has already been pointed out (1939), the numerical analysis can best be conducted by calculating the sums of squares for rows and columns freed from varietal differences, as this enables direct estimates to be made of the accuracy of the row and column comparisons.

If this is done, and at the same time the sum of squares for varieties is calculated directly from the varietal totals, the intra-block error may be obtained by subtraction.

The sum of squares for rows freed from varietal effects is given by the aggregate of the sums of squares of the deviations of each set of L 's. The divisor for this sum of squares is $\frac{1}{2}p(p^2-1)$. The sum of squares for columns freed from varietal effects is obtained similarly.

(d) *Calculation of the relative weights*

The yields, apart from varietal effects, may be regarded as the sum of three quantities normally and independently distributed, the first of which has the same value for all plots of a row, the second of which has the same value for all plots of a column, and the third of which varies from plot to plot. If the variance of the first quantity is A_r , second quantity A_c , and the third B , and there were no varietal effects to be eliminated, the expectation of the mean square for rows in the analysis of variance would be $pA_r + B$. Since the varietal effects corresponding to any set of rows are confounded in one out of $\frac{1}{2}(p+1)$ replications, the effect of the elimination of the varietal effects from rows is to reduce this expectation to $p \left\{ 1 - \frac{1}{\frac{1}{2}(p+1)} \right\} A_r + B$ or $p \frac{p-1}{p+1} A_r + B$. The expectation of the error mean square will be B .

If E_r' , E_c' and E_i are the mean squares for rows, columns and error respectively, and we equate these mean squares to their expectations, we have

$$1/w_r = pA_r + B = \{(p+1) E_r' - 2E_i\}/(p-1),$$

and similarly for $1/w_c$. Also $1/w_i = E_i$. We then find

$$\lambda = \frac{2(E_r' - E_i)}{(p-1) E_r'} \quad \text{and} \quad \mu = \frac{2(E_c' - E_i)}{(p-1) E_c'}.$$

When E_r' or E_c' is less than E_i , λ or μ , as the case may be, is taken to be zero, since it may be assumed that in no case are the inter-row or inter-column comparisons more accurate than the intra-row and column comparisons.

The determination of the relative weights is necessarily subject to errors of estimation, and this will lead to some loss of accuracy. The point is investigated further in § 6.

When λ and μ have been found, the adjusted yields can be calculated in the manner already indicated.

(e) Standard errors of the adjusted yields

Each pair of varieties has either a row in common or a column in common in one of the squares. If there is a row, say, in square I in common, the difference of the adjusted total yields is

$$\frac{1}{p} \{ ({}_wP_{2v} - {}_wP_{2v'}) + ({}_wP_{3v} - {}_wP_{3v'}) + \dots \\ + ({}_wP_{1'v} - {}_wP_{1'v'}) + ({}_wP_{2'v} - {}_wP_{2'v'}) + \dots \}$$

there being $\frac{1}{2}(p-1)$ differences corresponding to rows, and $\frac{1}{2}(p+1)$ differences corresponding to columns.

The variance of each of the row differences, $P_{2v} - P_{2v'}$, etc., is $\frac{\frac{1}{2}p(p+1)^2}{w_r + \frac{1}{2}(p-1)w_i}$, and of each of the column differences is $\frac{\frac{1}{2}p(p+1)^2}{w_c + \frac{1}{2}(p-1)w_i}$. Consequently the variance of the difference of the adjusted yields of the two varieties is

$$2 \times \frac{(p+1)^2}{4p} \left\{ \frac{\frac{1}{2}(p-1)}{w_r + \frac{1}{2}(p-1)w_i} + \frac{\frac{1}{2}(p+1)}{w_c + \frac{1}{2}(p-1)w_i} \right\}.$$

If the pair of varieties have a column in common, it is only necessary to interchange w_r and w_c in the above expression.

These two variances cannot differ very greatly, the maximum possible difference being $2/p^2 \times$ the mean variance of a difference. Consequently it will ordinarily be sufficient to take the mean variance as the common

variance of all comparisons. In terms of the quantities already given, this gives the following expression for the standard error of a single adjusted varietal total:

$$\sqrt{\frac{1}{2}} (p+1) \left\{1 + \frac{1}{2} (\lambda + \mu)\right\} E_i.$$

3. EXAMPLE OF 5×5 LATTICE WITH THREE REPLICATIONS

Table II gives the plan and yields in pounds of sugar of a sugar beet experiment conducted at Woburn in 1939. This was a manurial experiment which included the 25 treatment combinations shown in Table III. As the interactions were of particular interest, it was considered advisable to obtain all comparisons with equal accuracy, instead of adopting the usual procedure of confounding some of the higher order interactions, and a lattice square design was therefore adopted. Each plot was 65.2 links (along rows) by 25 links (along columns). The harvested area of each plot was $\frac{1}{75}$ acre.

The steps of the analysis are as follows:

(1) The row and column totals are obtained and entered in Table II, and the treatment totals are also obtained and entered in Table III.

(2) The quantities L and M are calculated and entered in Table II. Thus, for instance,

$$+17.1 = 177.5 + 207.9 + 212.6 + 195.9 + 189.5 - 3 \times 322.1,$$

with the check

$$-251.7 = 4857.9 - 3 \times 1703.2.$$

(3) The analysis of variance is completed as shown in Table IV. The total sum of squares and the sums of squares for squares and treatments are calculated in the ordinary manner. The sum of squares for rows is obtained from the sets of quantities L , with divisor $\frac{1}{4} \times 5 \times 24 = 30$, being

$$\frac{1}{30} (17.1^2 + 25.6^2 + \dots + 38.1^2 + \dots + 84.2^2 + \dots) \\ - \frac{1}{150} (251.7^2 + 104.7^2 + 147.0^2).$$

The sum of squares for columns is similarly obtained from the sets of quantities M . Finally, the error sum of squares is obtained by subtraction, and the mean squares are calculated.

(4) The quantities λ and μ are now calculated as follows:

$$\lambda = \frac{2 (84.94 - 16.88)}{4 \times 84.94} = 0.4006, \quad \frac{\lambda}{p} = 0.0801, \\ \mu = \frac{2 (26.20 - 16.88)}{4 \times 26.20} = 0.1779, \quad \frac{\mu}{p} = 0.0356.$$

Table II

Square I						Total	<i>L</i>	δ
<i>sy</i>	<i>ny</i>	<i>n</i>	<i>sz</i>	<i>s</i>				
61.2	67.7	68.1	65.2	59.9		322.1	+17.1	+1.4
<i>mz</i>	<i>nx</i>	<i>mx</i>	<i>nw</i>	<i>nz</i>				
69.4	67.4	64.9	77.6	67.7		347.0	+25.6	+2.1
<i>w</i>	<i>x</i>	<i>c</i>	<i>cy</i>	<i>m</i>				
46.3	59.7	76.2	80.3	71.2		333.7	-93.7	-7.5
—	<i>cx</i>	<i>my</i>	<i>cw</i>	<i>mw</i>				
55.2	77.0	82.4	80.4	77.7		372.7	-120.8	-9.7
<i>sw</i>	<i>sz</i>	<i>z</i>	<i>y</i>	<i>cz</i>				
73.9	79.0	51.6	44.8	78.4		327.7	-79.9	-6.4
Total	306.0	350.8	343.2	348.3	354.9	1703.2		-20.1
<i>M</i>	-39.2	-53.1	-40.3	-82.3	-36.8		-251.7	
ϵ	-1.4	-1.9	-1.4	-2.9	-1.3	-8.9		

Square II						Total	<i>L</i>	δ
<i>mz</i>	<i>y</i>	<i>c</i>	<i>s</i>	<i>cx</i>				
69.1	49.6	72.6	68.7	74.0		334.0	-38.1	-3.1
<i>cw</i>	<i>n</i>	<i>nz</i>	<i>w</i>	<i>cz</i>				
62.7	74.0	78.1	40.0	66.3		321.1	+3.8	+0.3
<i>ny</i>	<i>nz</i>	<i>sw</i>	<i>my</i>	<i>cy</i>				
68.9	78.1	69.6	68.1	68.5		353.2	+20.1	+1.6
<i>m</i>	—	<i>sz</i>	<i>sx</i>	<i>mx</i>				
64.7	38.6	57.4	65.5	67.1		293.3	+60.5	+4.9
<i>z</i>	<i>x</i>	<i>mw</i>	<i>nw</i>	<i>sy</i>				
48.0	39.0	64.9	72.2	58.7		282.8	+58.4	+4.7
Total	313.4	279.3	342.6	314.5	334.6	1584.4		+8.4
<i>M</i>	+23.9	+11.0	+10.2	+22.7	+36.9		+104.7	
ϵ	+0.9	+0.4	+0.4	+0.8	+1.3	+3.8		

Square III						Total	<i>L</i>	δ
<i>sy</i>	<i>my</i>	<i>nx</i>	<i>y</i>	<i>m</i>				
57.6	69.2	64.0	31.8	58.4		281.0	+84.2	+6.8
<i>nz</i>	<i>sz</i>	<i>z</i>	<i>w</i>	<i>cx</i>				
72.2	73.3	46.2	45.9	73.4		311.0	-16.7	-1.3
<i>sx</i>	<i>mz</i>	<i>cy</i>	<i>mw</i>	<i>n</i>				
61.4	77.9	73.1	70.4	70.5		353.3	+9.9	+0.8
<i>c</i>	<i>cz</i>	—	<i>ny</i>	<i>nw</i>				
58.6	68.4	46.7	71.3	69.1		314.1	+45.5	+3.6
<i>cw</i>	<i>x</i>	<i>s</i>	<i>mx</i>	<i>sw</i>				
56.6	52.9	60.9	71.8	68.7		310.9	+24.1	+1.9
Total	306.4	341.7	290.9	291.2	340.1	1570.3		+11.8
<i>M</i>	+89.3	-28.4	+34.5	+9.5	+42.1		+147.0	
ϵ	+3.2	-1.0	+1.2	+0.3	+1.5	+5.2		

Grand total=4857.9

The quantities *L* are multiplied by λ/p to give the δ 's, and the quantities *M* by μ/p to give the ϵ 's, which are entered in Table II.

(5) The adjusted total yields are then calculated by adding to each

unadjusted total yield the appropriate δ 's and ϵ 's, six in all. Thus the adjusted total yield of the unmanured plots is

$$140.5 - 9.7 - 1.4 + 4.9 + 0.4 + 3.6 + 1.2 = 139.5.$$

The standard error of each total is given by

$$\sqrt{[3 \times \{1 + \frac{1}{2} (0.4006 + 0.1779)\} \times 16.88]} = 8.08.$$

These yields have finally to be converted to cwt. per acre by multiplying by the conversion factor 0.2232. The converted yields are shown in Table VIII.

Table III. *Treatment totals*

	Nil	Nitrate of soda (n)	Calcium nitrate (c)	Sulphate of ammonia (s)	Muriate of ammonia (m)
Nil —	140.5	212.6	207.4	189.5	194.3
Pot. chloride (w)	132.2	218.9	199.7	212.2	213.0
Sod. chloride (x)	151.6	209.5	224.4	205.9	203.8
Pot. sulphate (y)	126.2	207.9	221.9	177.5	219.7
Sod. sulphate (z)	145.8	218.0	213.1	195.9	216.4

It is worth noting that in spite of the additional restrictions that have been imposed on the randomization the experiment can be validly analysed as if it were one in ordinary randomized blocks, the error sum of squares being simply the difference of the total sum of squares and the sums of squares for squares (blocks) and treatments in Table IV. This analysis gives the error appropriate to the unadjusted treatment totals. In this example the standard error is $\sqrt{(3 \times 36.22)} = 10.42$.

Table IV. *Analysis of variance*

	D.F.	Sum of squares	Mean square
Squares	2	426.33	213.16
Rows, eliminating treatments	12	1019.26	84.94
Columns, eliminating treatments	12	314.34	26.20
Treatments, ignoring rows and columns	24	7346.68	306.11
Error	24	405.04	16.88
Total	74	9511.65	

Since $10.42^2/8.08^2 = 1.66$, the gain in efficiency resulting from the adjustment, attributable to the use of lattice squares instead of ordinary randomized blocks, is 66% (excluding losses due to errors in weighting, discussed in § 6). In other words, to attain results of the same accuracy by the use of ordinary randomized blocks five replications would have been required instead of three.

Had the original method of complete elimination of rows and columns

been followed, the standard error of an adjusted treatment total would have been

$$\sqrt{(3 \times 16.88 \times 6/4)} = 8.72.$$

The gain in information over ordinary randomized blocks would therefore have been $10.42^2/8.72^2 - 1$ or 43%.

4. CASE OF $(p+1)$ REPLICATIONS

(a) *Estimation of varietal differences*

When there are $(p+1)$ replications the row and the column comparisons each give information on all the varietal degrees of freedom. It is therefore possible, by applying the inverse of the process outlined in § 2 (a), to construct estimates of the varietal differences from the row totals only, and from the column totals only. If ${}_r y_v$ and ${}_c y_v$ represent these estimates (in terms of the total yield of each variety), and $S(R_v)$ and $S(C_v)$ are respectively the sums of all the row and column totals containing variety v , then

$$p {}_r y_v = (p+1) S(R_v) - G, \quad p {}_c y_v = (p+1) S(C_v) - G.$$

The factor $(p+1)$ has to be included since each set of comparisons given by the row totals is based on only one of the $p+1$ replications.

If ${}_u y_v$ is the unadjusted estimate of the total yield of variety v , and ${}_i y_v$ is the estimate based on intra-row and column comparisons only, we have

$${}_u y_v = S(y_v) = \{ {}_r y_v + {}_c y_v + (p-1) {}_i y_v \} / (p+1).$$

The weighted estimate ${}_w y_v$ is therefore given by

$$\begin{aligned} {}_w y_v &= \frac{w_r {}_r y_v + w_c {}_c y_v + (p-1) w_i {}_i y_v}{w_r + w_c + (p-1) w_i} \\ &= {}_u y_v + \frac{w_i - w_r}{w_r + w_c + (p-1) w_i} ({}_u y_v - {}_r y_v) + \frac{w_i - w_c}{w_r + w_c + (p-1) w_i} ({}_u y_v - {}_c y_v) \\ &= {}_u y_v + \frac{\lambda'}{p} L_v' + \frac{\mu'}{p} M_v', \end{aligned}$$

where $L_v' = p ({}_u y_v - {}_r y_v) = p S(y_v) - (p+1) S(R_v) + G,$

$M_v' = p ({}_u y_v - {}_c y_v) = p S(y_v) - (p+1) S(C_v) + G,$

$$\lambda' = \frac{w_i - w_r}{w_r + w_c + (p-1) w_i}, \quad \mu' = \frac{w_i - w_c}{w_r + w_c + (p-1) w_i}.$$

(b) The analysis of variance

The sum of squares for rows freed from varietal but not column effects is given by the sum of squares of the quantities L_v' already obtained, the divisor being $p^3(p+1)$, and similarly the sum of squares for columns freed from varietal but not from row effects is given by the sum of squares of the quantities M_v' .

In order to complete the analysis of variance it is also necessary to form estimates of the row differences freed from both varietal and column differences. In terms of our previous notation the row differences of square I (in terms of totals of p plots) are given by the differences of the p quantities

$${}_1P_{1v} - \frac{1}{p-1} ({}_{II}P_{1v} + {}_{III}P_{1v} + \dots + {}_{II}P_{1v} + {}_{III}P_{1v} + \dots).$$

These sets of differences can be combined in just the same manner as were the row totals. The total of $(p-1)$ times all the quantities corresponding to rows containing variety v (with sign reversed) will be found to be

$$J_v = p S(y_v) - p S(R_v) - S(C_v) + G.$$

The sum of squares of these p^2 quantities will give the sum of squares for rows freed from both varietal and column effects, the divisor being $p^3(p-1)$.

Similarly the sum of squares for columns freed from varietal and row effects is obtained from the quantities

$$K_v = p S(y_v) - S(R_v) - p S(C_v) + G.$$

We can therefore construct the analysis of variance table either by including rows eliminating both varieties and columns, and columns eliminating varieties only, or by including rows eliminating varieties only, and columns eliminating both rows and varieties. In practice it is best to calculate both sets of sums of squares, as these provide a useful check with very little extra labour.

(c) Calculation of the relative weights, etc.

In adjusting the row differences of any one square, so as to free them from varietal effects without introducing any column effects, there are $p-1$ squares available for determining the varietal effects, since in the remaining square the varietal effects involved are confounded with columns. Consequently the expectation of the mean square for rows, eliminating varieties and columns, is reduced from $pA_r + B$ to $(p-1)A_r + B$.

Equating this expectation to the actual mean square E_r' , we find

$$w_r = \frac{p-1}{pE_r' - E_i}$$

Similarly

$$w_c = \frac{p-1}{pE_c' - E_i}$$

and $w_i = 1/E_i$.

As before, if E_r' or E_c' is less than E_i , w_r or w_c may be taken to be equal to w_i .

Nothing is gained by substituting for w_r , w_c and w_i in the expressions for λ' and μ' , which are best evaluated by first determining w_r , w_c and w_i .

The standard error of the adjusted total yields is

$$\sqrt{(p+1)(1+\lambda'+\mu')E_i}.$$

5. EXAMPLE OF 5×5 LATTICE WITH SIX REPLICATIONS

An experiment similar to that described in § 3, but with six replications, was carried out at Rothamsted in 1939. To save space the plan and the individual plot yields, and also some of the details of the computations, have not been reproduced here, but Tables V and VI give the necessary totals from which the remainder of the analysis can be completed.

Table V. *Treatment totals*

		<i>n</i>	<i>c</i>	<i>s</i>	<i>m</i>
—	297.3	341.7	351.5	330.9	358.0
<i>w</i>	289.4	356.4	362.1	333.2	344.7
<i>x</i>	292.9	363.9	366.5	334.7	354.1
<i>y</i>	308.7	363.9	338.3	341.1	330.4
<i>z</i>	283.5	369.7	359.0	349.6	357.9

Table VI. *Sums of row and column totals*

<i>S (R_v)</i>					
		<i>n</i>	<i>c</i>	<i>s</i>	<i>m</i>
—	1691.2	1677.1	1691.5	1661.1	1745.1
<i>w</i>	1640.4	1709.5	1773.8	1723.8	1693.3
<i>x</i>	1673.5	1694.2	1750.3	1628.7	1679.0
<i>y</i>	1721.0	1735.1	1687.1	1677.9	1660.0
<i>z</i>	1619.7	1761.9	1722.4	1688.9	1690.5
<i>S (C_v)</i>					
		<i>n</i>	<i>c</i>	<i>s</i>	<i>m</i>
—	1644.2	1711.3	1734.1	1712.9	1722.1
<i>w</i>	1649.3	1716.7	1710.4	1659.1	1650.4
<i>x</i>	1687.2	1762.8	1729.5	1676.2	1739.2
<i>y</i>	1670.9	1684.4	1682.6	1720.2	1688.3
<i>z</i>	1651.7	1677.3	1696.4	1701.5	1718.3

The steps of the analysis are as follows:

(1) The row and column totals of each square are entered in the original table of yields in their appropriate positions.

(2) The treatment totals, and the totals of all the row totals containing a given treatment, and of all the column totals containing a given treatment, are obtained. These are shown in Tables V and VI. Thus the totals of the rows containing the untreated plots in the six squares are 258.1, 277.4, 291.7, 278.1, 302.5 and 283.4, giving a total of 1691.2.

As a good deal of the labour involved in the construction of these tables consists of locating the plots having the given treatment, it is worth while to form all three totals simultaneously. This can be done on an ordinary calculating machine by shifting the carriage.

(3) The quantities L' , M' , J and K can now be calculated. To facilitate this calculation it is worth tabulating the differences D of the sums of the row totals and the sums of the column totals. The four quantities can then be computed successively with great rapidity. The calculation for the no fertilizer treatment proceeds as follows:

$$D_0 = S(R_0) - S(C_0) = 1691.2 - 1644.2 = +47.0,$$

$$L'_0 = 5S(y_0) - 6S(R_0) + G = 5 \times 297.3 - 6 \times 1691.2 + 8479.4 = -181.3,$$

$$J_0 = L'_0 + D_0 = -181.3 + 47.0 = -134.3,$$

$$K_0 = J_0 + 4D_0 = -134.3 + 4 \times 47.0 = +53.7,$$

$$M'_0 = K_0 + D_0 = +53.7 + 47.0 = +100.7,$$

with the check

$$M'_0 = 5 \times 297.3 - 6 \times 1644.2 + 8479.4 = +100.7.$$

The numerical values of L' , M' , J and K are set out in four tables of 25 values each, similar to Tables V and VI. The sum of all the values in each of these four tables should be zero.

(4) The analysis of variance is next completed, as shown in Table VII. The total sum of squares and the sums of squares for squares and treatments are calculated in the ordinary manner. The sum of squares for rows, eliminating varieties, is given by the sum of the squares of all the values of L' , with divisor $5^3 \times 6 = 750$. The sum of squares for columns, eliminating varieties, is similarly obtained from the values of M' . The sum of squares for rows, eliminating varieties and columns, is obtained from the values of J , with divisor $5^3 \times 4 = 500$, and the sum of squares for columns, eliminating varieties and rows, from the values of K . In no case is there any correction to be deducted from these sums of squares, since each set of values has zero total.

Finally the error sum of squares is obtained by subtraction, including either rows eliminating varieties only, and columns eliminating both varieties and rows, or alternatively rows eliminating both varieties and columns, and columns eliminating varieties only. This provides a valuable check.

Table VII. *Analysis of Variance*

	D.F.	Sum of squares	Mean square
Squares	5	350.28	70.06
Rows, eliminating treatments	24	1074.43	44.77
Rows, eliminating treatments and columns	24	1036.58	43.19
Columns, eliminating treatments	24	664.95	27.71
Columns, eliminating treatments and rows	24	627.10	26.13
Treatments, ignoring rows and columns	24	2658.03	110.75
Error	72	915.55	12.72
Total	149	5625.39	

(5) The quantities w_r , w_c , w_i , λ' and μ' are then calculated as follows:

$$w_r = \frac{4}{5 \times 43.19 - 12.72} = 0.01968,$$

$$w_c = \frac{4}{5 \times 26.13 - 12.72} = 0.03392,$$

$$w_i = 1/12.72 = 0.07862,$$

$$w_i - w_r = 0.05894, \quad w_i - w_c = 0.04470, \quad w_r + w_c + 4w_i = 0.36808,$$

$$\lambda' = 0.1601, \quad \frac{1}{3}\lambda' = 0.03202,$$

$$\mu' = 0.1214, \quad \frac{1}{3}\mu' = 0.02428.$$

(6) The adjusted total yields are calculated by multiplying L' and M' by λ'/p and μ'/p respectively, and adding these corrections to the unadjusted total yields. Thus for the no fertilizer treatment the adjusted yield is

$$297.3 + 0.03202 \times (-181.3) + 0.02428 \times (+100.7) = 293.9.$$

The standard error of each adjusted yield is given by

$$\sqrt{\{6(1 + 0.1601 + 0.1214)12.72\}} = 9.89.$$

The yields are then converted to cwt. per acre (conversion factor 0.1339), these converted yields being shown for both experiments in Table VIII.

There is no need to discuss the results of the experiments in detail here. The responses to the mineral salts were only small, and consequently it is not to be expected that any very striking interactions with the

nitrogenous fertilizers will be revealed. A small response to the mineral fertilizers containing sodium is shown in both experiments, and reaches significance in the Woburn experiment. Nitrate of soda is also the best of the nitrogenous fertilizers in both experiments, being significantly better than sulphate of ammonia at Rothamsted and both sulphate of ammonia and calcium nitrate at Woburn. The only apparent interaction between the mineral and nitrogenous fertilizers is a depression with potash salts in the absence of nitrogen at Woburn.

Table VIII. *Adjusted yields, cwt. per acre, Rothamsted and Woburn*

	Rothamsted						Woburn					
		Sod. nitr.	Calc. nitr.	Amm. sulph.	Amm. chl.	Mean		Sod. nitr.	Calc. nitr.	Amm. sulph.	Amm. chl.	Mean
—	39.4	46.0	46.9	44.6	46.8	44.8	31.1	48.1	45.2	42.5	44.6	42.3
Pot. chl.	39.2	47.7	47.1	44.4	47.3	45.2	27.5	51.0	43.2	46.8	46.5	43.0
Sod. chl.	38.3	48.4	48.1	46.8	47.6	45.8	33.1	48.7	47.1	46.3	47.5	44.6
Pot. sulph.	40.0	48.9	45.7	45.7	45.0	45.1	27.1	47.3	48.3	43.2	48.4	42.8
Sod. sulph.	38.7	49.3	48.1	47.3	48.4	46.4	32.0	49.7	46.8	44.1	47.9	44.1
Mean	39.1	48.1	47.2	45.7	47.0	45.4	30.2	49.0	46.1	44.6	47.0	43.4
St. error				1.32						1.80		

If the Rothamsted experiment is analysed as if it were one in ordinary randomized blocks, the standard error of an unadjusted treatment total is found to be 11.44. Thus the gain in efficiency resulting from the adjustment, attributable to the use of lattice squares instead of ordinary randomized blocks, is 34 %. In other words, to obtain results of the same accuracy by the use of ordinary randomized blocks, eight replications would have been required instead of six.

Had the original method of complete elimination of rows and columns been followed, the standard error of an adjusted treatment total would have been 10.70. The gain in information over ordinary randomized blocks would therefore have been 14 %. The new method of adjustment has therefore resulted in a further gain of about 18 %, less errors due to inaccuracies of weighting, which, as shown in the next section, are not likely to be more than about 3 %.

6. LOSS OF INFORMATION DUE TO INACCURACIES OF WEIGHTING

Since the relative weights are estimated from somewhat small numbers of degrees of freedom, it is important to ascertain whether the loss of information due to inaccuracies of weighting is so great as to nullify the gain in accuracy resulting from the weighting.

In the case of $\frac{1}{2}(p+1)$ replications the degrees of freedom confounded

with rows may be considered separately from those confounded with columns. We then obtain the following general expression for the percentage loss of information on the comparisons confounded with either rows or columns

$$\frac{(2\rho+1)(F-1)^2}{\{(2\rho+1)F-1\}^2+2\rho},$$

where ρ is the ratio of the true intra-row and column weight to the true inter-row or inter-column weight, and F is distributed as is e^{2z} with $\frac{1}{2}(p^2-1)$ and $\frac{1}{2}(p-3)(p^2-1)$ degrees of freedom.

For any given value of ρ a series of values of this expression may be calculated, corresponding to the various probability levels of the F distribution. For probability levels which would give $w_i < w_r$ or $w_i < w_c$ the value of F which gives $w_i = w_r$ or $w_i = w_c$ is taken. The average loss of information may then be obtained by numerical integration over the whole probability scale.

The case of $p=5$ with three replications has been worked out, the following losses of information being obtained:

ρ	1	2	4	6	8	12
Mean percentage loss				2.52	4.04	4.02	3.14	2.53	1.81

These losses must be set off against the gains resulting from the use of the lattice squares. If the amount of information obtained from ordinary randomized blocks of 25 plots is taken as a 100 units, we obtain the following table of values for the net amount of information obtainable from the use of lattice squares:

ρ	1	2	4	6	8	12
Information with known weights						100.0	111.1	150.0	192.6	236.1	324.1
Loss due to inaccuracies of weights						2.5	4.5	6.0	6.1	6.0	5.9
Net information						97.5	106.6	144.0	186.5	230.1	318.2

It thus appears that even in a set of three 5×5 lattice squares, where only 12 degrees of freedom are available for estimating the accuracy of the row or column comparisons, the loss of information due to inaccuracies of weighting is by no means sufficiently great to outweigh the advantages of the lattice design. With a larger number of replications, or with larger squares, the loss of information from this cause will be quite trivial.

SUMMARY

A new method of analysing the results of experiments arranged in lattice (quasi-Latin) square designs is described. By this method the information contained in the inter-row and inter-column comparisons

is recovered. The procedure followed is to estimate the actual accuracy of the inter-row and inter-column comparisons relative to intra-row and column comparisons, and assign weights proportional to these relative accuracies.

The methods of computation by which this procedure can be carried out expeditiously are described in detail, and are illustrated by two experiments carried out at Rothamsted and Woburn. The gains in accuracy obtained in these two experiments (as compared with ordinary randomized blocks) were approximately 30% and 60% respectively, allowing for losses of information due to inaccuracies of weighting.

These gains have been obtained without any additional field work, the only extra work being that required in drawing up the arrangement and in analysing the results. Moreover, if for any reason the additional statistical analysis cannot be undertaken, or does not appear to be worth while, lattice square experiments can be validly analysed just as if they were arrangements in ordinary randomized blocks.

It appears, therefore, that the use of lattice squares is likely to be of considerable value in variety trials involving large numbers of varieties, especially in those cases where ordinary Latin squares are found to be effective in reducing the variability of the experimental material.

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THE RECOVERY OF INTER-BLOCK INFORMATION IN BALANCED INCOMPLETE BLOCK DESIGNS

By F. YATES

1. INTRODUCTION

INCOMPLETE block and quasi-factorial designs of various kinds were first introduced by the author a few years ago (1936*a, b*). All these designs have the property that the number of varieties (or treatments) included in each block is smaller than the total number to be tested. There is consequently a gain in precision due to the use of smaller blocks, at the expense of loss of information on those varietal comparisons which are confounded with blocks. In the original papers only the complete elimination of inter-block differences was considered. These inter-block comparisons will, however, contain an appreciable amount of information, amounting in the limiting case, when the inter-block and intra-block comparisons are of equal accuracy, to a fraction $1 - E$ of the total information, where E is the efficiency factor.

The recovery of this information has already been discussed for three-dimensional quasi-factorial designs (1939) and also for lattice (quasi-Latin) squares (1940). The present paper contains a similar discussion of balanced incomplete block designs. The case of two-dimensional quasi-factorial designs is to be dealt with in a publication by the Statistical Department of Iowa State College.

2. ESTIMATES OF THE VARIETAL DIFFERENCES

If r varieties are arranged in b blocks containing k varieties each, there being r replicates of each variety, the condition of balance will be fulfilled if each pair of varieties occurs together an equal number λ of times. A catalogue of possible designs and known solutions is given by Fisher & Yates (1938).

The following relations hold:

$$rb = kr,$$

$$(r-1)\lambda = (k-1)r.$$

The efficiency factor E , defined as the fraction of the total information contained in the intra-block comparisons, when inter-block and intra-block comparisons are of equal accuracy, is given by

$$E = \frac{1-1/k}{1-1/r} = \frac{r\lambda}{rk}.$$

Let V_s be the sum of all the yields of variety s , T_s the sum of all the block totals of blocks containing variety s , T'_s the sum of all the remaining block totals, and G the total yield of all plots. Then, as has been shown previously (1936*a*), the estimates of the varietal differences derived from the intra-block comparisons are obtained from the quantities

$$Q_s = V_s - T_s/k,$$

or, as is more convenient when $k > \frac{1}{2}v$,

$$Q'_s = V_s + T'_s/k.$$

The actual differences in units of the total yield of the r replicates are given by the differences of

$$Q_s/E \quad \text{or} \quad Q'_s/E,$$

the sum of the first set being zero, and the second set $rG/\lambda v$.

The error variance of these latter sets of quantities is r/Ew , where $1/w$ is the intra-block error variance.

The estimates of the varietal differences derived from the inter-block comparisons are similarly given by the differences of

$$rT_s/(r-\lambda) \quad \text{or} \quad rT'_s/(r-\lambda),$$

in units of the total yield of r replicates. The error variance of these sets of quantities is $kr^2/(r-\lambda)w'$, where $1/w'$ is the error variance of the inter-block comparisons, in units of a single plot.

If the weights w and w' are known, the most efficient estimates of the varietal differences will be given by the differences of the weighted means:

$$Y_s = \frac{\frac{Q_s}{E} \frac{Ew}{r} + \frac{rT_s}{r-\lambda} \frac{(r-\lambda)w'}{kr^2}}{\frac{Ew}{r} + \frac{(r-\lambda)w'}{kr^2}}.$$

The quantities Y_s may be termed the partially adjusted yields (totals of r replicates). If G is the total yield of all plots, Y_s may be written (after the addition of a quantity $\mu(k-1)G$) in the form

$$Y_s = V_s + \mu W_s,$$

where

$$W_s = (v-k)V_s - (v-1)T_s + (k-1)G,$$

and

$$\mu = \frac{w-w'}{wv(k-1) + w'(v-k)}.$$

The error variance of the Y 's is

$$\frac{1}{\frac{Ew}{r} + \frac{(r-\lambda)w'}{kr^2}} = \frac{kr(r-1)}{wv(k-1) + w'(v-k)}.$$

3. THE ANALYSIS OF VARIANCE

The structure of the analysis of variance is a little complicated. The residual sum of squares for intra-block error may be calculated (*a*) by deducting the sum of squares for blocks (ignoring varieties) and for varieties (eliminating blocks) from the total sum of squares, or alternatively (*b*) by deducting the sum of squares for varieties (ignoring blocks) and for blocks (eliminating varieties).

As has previously been shown, the sum of squares for varieties (eliminating blocks) is derived from the sum of the squares of the quantities Q , with divisor rE .

The sum of squares for blocks (eliminating varieties) splits into two parts. The first, corresponding to $v-1$ degrees of freedom, is affected by varietal differences and is derived from the sum of the squares of the deviations of the quantities W_g , with divisor $rv(v-k)(k-1)$. The second, corresponding to $b-v$ degrees of freedom, is unaffected by varietal differences, and represents pure inter-block error. This latter sum of squares can best be computed by taking the difference of the total sum of squares for blocks (ignoring varieties) and the component of this sum of squares which is affected by varietal differences, this being given by the sum of squares of the deviations of T_g , with divisor $k(r-\lambda)$.

Table 1 shows these relations in tabular form. In this table dev^2 indicates the sum of the squares of the deviations, y the individual yields and B the block totals. By calculating both forms of the analysis a complete check is obtained, except for the total sum of squares and for the total sum of squares for blocks (ignoring varieties).

Table 1. *Structure of analysis of variance*

Method (a)	D.F.	S.S. (a)	S.S. (b)	Method (b)
Blocks (ignoring varieties):				Blocks (eliminating varieties):
Varietal component	$v-1$	$\frac{dev^2 T}{k(r-\lambda)}$	$\frac{dev^2 W}{rv(v-k)(k-1)}$	Varietal component
Remainder	$b-v$	\dagger	\dagger	Remainder
Total	$b-1$	$\frac{dev^2 B^*}{k}$	\dagger	Total
Varieties (eliminating blocks)	$v-1$	$\frac{dev^2 kQ}{k^2 rE}$	$\frac{dev^2 V}{r}$	Varieties (ignoring blocks)
Intra-block error	$rv-v-b+1$	\dagger	\dagger	Intra-block error
Total	$rv-1$	$dev^2 y^*$	$dev^2 y^*$	Total

* Requires checking.

† Calculated by addition or subtraction.

4. ESTIMATION OF THE RELATIVE WEIGHTS

If the intra-block error variance is B , and the error variance of block totals is $k(kA+B)$, the expectations of the mean squares corresponding to the components of the sum of squares for varieties (eliminating blocks) are shown in Table 2.

Table 2. *Expectations of mean squares for blocks (eliminating varieties)*

	D.F.	Expectation
Varietal component	$v-1$	$E k A + B$
Remainder	$b-v$	$k A + B$
Total	$b-1$	$\frac{bk-v}{b-1} A + B$

The factor E in the first of the above expressions is derived as follows. If for any pair of varieties s and s' the coefficients of each plot yield in the difference $W_s - W_{s'}$ are written

down, and summed by blocks, it will be found that $(r-\lambda)$ of these sums equal $v(k-1)$ and $(r-\lambda)$ equal $-v(k-1)$, the remainder being zero. Utilizing the divisor given in Table 1 (which is itself one-half the sum of the squares of these coefficients), we obtain as the coefficient of A

$$\frac{(r-\lambda)v^2(k-1)^2}{rv(v-k)(k-1)} = Ek.$$

As has been pointed out previously (Yates, 1939), it is sufficient, for the purpose of estimating w and w' , to equate the expectation in terms of w and w' of the mean square for all the $b-1$ degrees of freedom for blocks (eliminating varieties) with the actual mean square M'' , say. If the mean square for intra-block error is M , we obtain the equations

$$w = \frac{1}{M}, \quad w' = \frac{v(r-1)}{k(b-1)M'' - (r-k)M}.$$

Since w may ordinarily be assumed to be greater than w' it will be sufficient, if M'' is less than M , to take w' as equal to w , i.e. to use the unadjusted yields as the final estimates.

Since M'' is frequently based on a somewhat small number of degrees of freedom, there is of course some inaccuracy in the estimated weights. The effect of this inaccuracy on the accuracy of the weighted estimates has been investigated in various extreme cases (Yates, 1939, 1940; and Cochran, unpublished material). The results obtained are summarized in Table 3.

Table 3. *Loss of information due to inaccuracies of weighting*

(a) Particulars of cases investigated

Case	Type of design	Replications	Degrees of freedom		Expectation of block m.s.		Efficiency factor	Reference to literature
			Blocks	Error	Actual	Unconfounded		
1	5×5 lattice	2	8	16	$\frac{2}{3}A+B$	$5A+B$	0.75	Cochran (unpublished)
2	4×4 triple lattice	3	9	21	$\frac{3}{4}A+B$	$4A+B$	0.769	Cochran (unpublished)
3	$3 \times 3 \times 3$ lattice	3	24	28	$2A+B$	$3A+B$	0.591	Yates (1939)
4	5×5 lattice squares	3	12	24	$\frac{10}{3}A+B$	$5A+B$	0.667	Yates (1940)

(b) Percentage losses of information for various values of w/w'

w/w'	1	2	4	6	8	12
Case 1	2.21	3.07	4.54	4.37	3.91	—
2	1.73	3.00	3.73	3.19	—	—
3	1.71	2.68*	2.54*	—	—	—
4	2.52	4.04	4.02	3.14	2.53	1.81

* These values are approximate only, being calculated on the assumption that the 24 degrees of freedom for blocks are homogeneous, with mean square expectation $2A+B$.

The actual loss of efficiency depends not only on the numbers of degrees of freedom for M'' and M , but also on the efficiency factor. From the cases already investigated, however, it may be concluded that this source of loss is of little importance in cases likely to occur in practice.

5. MODIFICATION WHEN GROUPS OF BLOCKS FORM COMPLETE REPLICATIONS

In certain cases the structure of the design is such that blocks fall into groups containing one or more complete replications of all the varieties. When this is so it is clearly advisable in agricultural trials to arrange such groups of blocks in compact large blocks on the ground, since the variation affecting the inter-block varietal estimates will thereby be reduced. Allowance for this must be made in the analysis of variance given above by eliminating complete replications (or groups of replications) from the remainder component of blocks. If there are c such large blocks (containing r/c replications each), the expectations given in Table 2 will require modification as in Table 4.

Table 4. *Expectations of mean squares when groups of blocks contain complete replications*

	D.F.	Expectation
Groups of blocks	$c - 1$	—
Varietal component	$c - 1$	$E k A + B$
Remainder	$b - c - c + 1$	$k A + B$
Varietal component + remainder	$b - c$	$\frac{b k - c - k(c - 1)}{b - c} A + B$

The formula for w' will also require modification, being in fact

$$w' = \frac{v(r-1) - k(c-1)}{k(b-c)M'' - (r-k)M}$$

In the common case in which each large block contains a single replication, $c = r$, and the expectation of the mean square for the $b - r$ degrees of freedom is $\frac{k(r-1)}{r} A + B$, the formula for w' being

$$w' = \frac{r-1}{rM'' - M}$$

6. SIMPLIFICATION WHEN $b = r$, AND WHEN $v = k^2$

When $b = r$ the analysis of variance reduces to the simplified form given in Table 5.

Table 5. *Analysis of variance when $b = r$*

	D.F.	S.S.
Blocks (eliminating varieties)	$c - 1$	$\frac{\text{dev}^2 W}{rv(v-k)(k-1)}$
Varieties (ignoring blocks)	$v - 1$	$\frac{\text{dev}^2 V}{r}$
Intra-block error	$(k-2)v + 1$	\dagger
Total	$kv - 1$	$\text{dev}^2 y$

There is little point in tabulating the Q 's, though they will provide a general check, as before, if this is desired.

A similar simplification is possible in the series of designs $v = k^2$, $r = k + 1$, $b = k(k + 1)$ (balanced lattices), where the remaining k degrees of freedom for blocks correspond to the contrasts of complete replications.

7. FIRST EXAMPLE

An example of a dummy trial of nine treatments (e.g. dietary treatments) superimposed on the scores of eighteen litters of four rats in a discrimination test is given by Fisher & Yates (1938). Here $v = 9$, $r = 8$, $k = 4$, $b = 18$, $\lambda = 3$, $E = 27/32$.

The individual scores have been given in the publication referred to. Table 6 shows the values of V , T , $4Q$ and W for the nine treatments $a-i$. The analysis of variance is shown in Table 7, which corresponds in arrangement to Table 1.

Table 6. Calculation of adjusted scores in discrimination test

	V	T	$4Q$ $= 4V - T$	W $= 5V - 8T + 3G$	Y $= V + \mu W$
a	43.9	152.2	+ 23.4	+ 65.1	45.7
b	39.1	156.4	0	+ 7.5	39.3
c	41.3	169.6	- 4.4	- 87.1	38.9
d	43.6	151.7	+ 22.7	+ 67.6	45.4
e	41.7	159.2	+ 7.6	- 1.9	41.6
f	35.6	162.0	- 19.6	- 54.8	34.1
g	28.6	138.3	- 23.9	+ 99.8	31.3
h	42.8	172.5	- 1.3	- 102.8	40.0
i	37.8	155.7	- 4.5	+ 6.6	38.0
	354.4	1417.6	0	0	354.3
Divisor	8	4.5 = 20	$4^2 \cdot 8 \cdot 27/32$ = 108	$8 \cdot 9 \cdot 5 \cdot 3$ = 1080	

Table 7. Analysis of variance, discrimination test

	D.F.	S.S. (a)	S.S. (b)	M.S. (b)
Blocks:				
Varietal component	8	41.4684	37.0634	4.6329
Remainder	9	138.2011	138.2011	15.3557
Total	17	179.6695	175.2645	10.3097
Varieties	8	19.6044	24.0094	—
Error	46	119.4506	119.4506	2.5968
Total	71	318.7245	318.7245	—

From the results of the analysis of variance we obtain

$$w = \frac{1}{2.5968} = 0.3851, \quad w' = \frac{63}{68 \times 10.3097 - 5 \times 2.5968} = 0.0916,$$
$$\mu = \frac{0.3851 - 0.0916}{27 \times 0.3851 + 5 \times 0.0916} = \frac{0.2935}{10.8557} = 0.02704.$$

The final adjusted scores in terms of the total scores of eight rats are given in the last column of Table 6. The standard error of these scores is

$$\sqrt{\frac{256}{10.8557}} = \sqrt{23.58} = 4.86.$$

The standard error of the completely adjusted scores (which are equal to Q/E) is

$$\sqrt{(8 \times 2.5968 \times 27/32)} = \sqrt{24.62} = 4.96.$$

Thus the gain in information from the recovery of the inter-block information is $24.62/23.58 - 1$ or 4.4 % (excluding losses due to inaccuracy of weighting). If inter-litter and intra-litter comparisons had been of equal accuracy, the gain would have been 18.5 %.

8. SECOND EXAMPLE

Table 8 gives the arrangement and yields of a tomato trial of 21 varieties arranged in twenty-one blocks of five plots. (I am indebted to the Statistical Department of Iowa State College for the data of this example.)

Table 8. *Arrangement and yields of a tomato variety trial*

Block ...	1	2	3	4	5	6	7						
<i>s</i>	22.25	<i>m</i>	32.00	<i>e</i>	51.75	<i>r</i>	45.75	<i>g</i>	49.25	<i>j</i>	59.00	<i>b</i>	61.25
<i>b</i>	51.50	<i>l</i>	44.00	<i>i</i>	58.50	<i>c</i>	37.25	<i>a</i>	33.75	<i>u</i>	72.50	<i>p</i>	47.75
<i>o</i>	41.56	<i>j</i>	52.50	<i>k</i>	29.75	<i>k</i>	17.50	<i>f</i>	45.75	<i>o</i>	49.50	<i>l</i>	45.00
<i>g</i>	36.75	<i>a</i>	50.75	<i>n</i>	70.75	<i>q</i>	26.75	<i>h</i>	55.25	<i>e</i>	46.50	<i>t</i>	35.00
<i>k</i>	21.00	<i>k</i>	32.25	<i>t</i>	56.00	<i>h</i>	37.25	<i>i</i>	62.80	<i>h</i>	78.00	<i>h</i>	53.00
Total	173.06	211.50	266.75	164.50	246.80	305.50	242.00						
Block ...	8	9	10	11	12	13	14						
<i>e</i>	31.25	<i>u</i>	51.00	<i>k</i>	24.75	<i>n</i>	55.25	<i>d</i>	36.50	<i>o</i>	38.75	<i>s</i>	28.00
<i>c</i>	35.25	<i>r</i>	49.00	<i>f</i>	47.25	<i>o</i>	37.75	<i>t</i>	43.50	<i>t</i>	42.25	<i>q</i>	40.50
<i>a</i>	40.50	<i>a</i>	40.50	<i>u</i>	50.50	<i>a</i>	39.50	<i>q</i>	35.25	<i>c</i>	42.50	<i>l</i>	50.25
<i>b</i>	58.50	<i>t</i>	47.75	<i>p</i>	58.75	<i>q</i>	46.75	<i>g</i>	44.00	<i>f</i>	50.25	<i>f</i>	62.50
<i>d</i>	45.50	<i>s</i>	38.50	<i>d</i>	51.25	<i>p</i>	48.25	<i>j</i>	51.75	<i>m</i>	30.75	<i>e</i>	41.00
Total	211.00	226.75	232.50	227.50	211.00	204.50	222.25						
Block ...	15	16	17	18	19	20	21						
<i>i</i>	65.00	<i>n</i>	57.50	<i>g</i>	67.00	<i>f</i>	74.00	<i>b</i>	67.00	<i>m</i>	44.75	<i>j</i>	74.25
<i>d</i>	47.50	<i>g</i>	55.00	<i>r</i>	70.50	<i>b</i>	68.25	<i>u</i>	56.75	<i>d</i>	62.00	<i>p</i>	68.50
<i>o</i>	49.75	<i>u</i>	55.50	<i>m</i>	46.00	<i>r</i>	86.00	<i>i</i>	66.00	<i>n</i>	76.75	<i>c</i>	46.25
<i>l</i>	51.00	<i>c</i>	38.75	<i>e</i>	43.00	<i>n</i>	93.25	<i>m</i>	31.75	<i>s</i>	46.75	<i>s</i>	50.25
<i>r</i>	64.50	<i>l</i>	51.25	<i>p</i>	64.50	<i>j</i>	98.12	<i>q</i>	49.00	<i>h</i>	82.25	<i>i</i>	65.50
Total	277.75	258.00	291.00	419.62	270.50	312.50	304.75						

Here $v = 21$, $r = 5$, $k = 5$, $b = 21$, $\lambda = 1$, $E = 21/25$. The values of V , T , W , and the adjusted yields are shown in Table 9, and the analysis of variance in Table 10.*

* Labour would have been saved had the yields been rounded off to 1 decimal place before analysis. The fact that three, and only three, of the yields are not exact quarters may also point to the existence of certain errors of transcription.

INCOMPLETE BLOCK DESIGNS

Table 9. *Calculation of the adjusted yields, tomato trial*

	V	T	W $= 16V - 20T + 4G$	Y $= V + \mu W$
<i>a</i>	205.00	1123.55	+ 1927.92	225.57
<i>b</i>	306.50	1316.18	- 300.68	303.29
<i>c</i>	200.00	1142.75	+ 1463.92	215.62
<i>d</i>	242.75	1244.75	+ 107.92	243.90
<i>e</i>	213.50	1296.50	- 1395.08	198.62
<i>f</i>	279.75	1325.67	- 918.48	269.95
<i>g</i>	252.00	1179.86	+ 1553.72	268.58
<i>h</i>	305.75	1271.30	+ 584.92	311.99
<i>i</i>	317.80	1366.55	- 1127.28	305.77
<i>j</i>	335.62	1452.37	- 2558.56	308.32
<i>k</i>	125.25	1048.31	+ 2156.72	148.26
<i>l</i>	241.50	1211.50	+ 752.92	249.53
<i>m</i>	185.25	1290.00	- 1717.08	166.93
<i>n</i>	353.50	1484.37	- 2912.48	322.43
<i>o</i>	217.31	1188.31	+ 829.68	226.17
<i>p</i>	287.75	1297.75	- 232.08	285.27
<i>q</i>	198.25	1095.75	+ 2375.92	223.60
<i>r</i>	315.75	1379.62	- 1421.48	300.59
<i>s</i>	185.75	1239.31	- 695.28	178.33
<i>t</i>	224.50	1151.00	+ 1690.92	242.54
<i>u</i>	286.25	1293.25	- 166.08	284.48
	5279.73	26398.65	0	5279.74
Divisor		5	5.21.16.4 = 6720	

Table 10. *Analysis of variance, tomato trial*

	D.F.	S.S.	M.S.
Blocks (eliminating varieties)	20	7105.99	355.30
Varieties (ignoring blocks)	20	14222.31	—
Error	64	2363.20	36.92
Total	104	23691.50	—

From Table 10 we have

$$w = 0.02709, \quad w' = \frac{84}{100 \times 355.30 - 16 \times 36.92} = 0.00240,$$

$$\mu = \frac{w - w'}{84w + 16w} = \frac{0.02469}{2.314} = 0.01067.$$

The standard error of the adjusted yields is $\sqrt{500/2.314} = \sqrt{216.1} = 14.70$. The standard error of the fully adjusted yields would be $\sqrt{219.8}$, so that the gain in information from the use of the inter-block information is trivial. If the inter-block and intra-block comparisons were of equal accuracy the gain would be 19.1 %, less losses due to inaccuracies of weighting.

9. GENERAL REMARKS

In both the examples given the gain in information due to the recovery of the inter-block information is small. Cases will arise, however, in which the chosen blocks do not account for much of the general variability, and in such cases the recovery of the inter-block

information will lead to an appreciable increase in efficiency. Since this recovery involves little additional work, and the resulting gain cannot in any case be assessed until the analysis of variance (on the lines set out in this paper) is performed, it would appear best to follow this method of analysis in all cases.

In agricultural experiments, however, the gains from the use of inter-block information will not in general be so great as in similar quasi-factorial (lattice) designs, since complete replications cannot (except in special cases) be arranged in compact groups of blocks. For this reason also, cases will arise in which the use of ordinary randomized blocks will be more efficient than the use of incomplete blocks, whereas lattice designs can never be less efficient than ordinary randomized blocks. Nor is it at all easy, except in data from uniformity trials, to determine exactly what is the efficiency of an incomplete block design, relative to an arrangement in ordinary randomized blocks on the same land.

It will be remembered that lattice designs can be analysed as if they were arrangements in ordinary randomized blocks, the errors of the unadjusted yields being correctly estimated by this process. This property does not hold for incomplete block designs (except those which can be arranged in complete replications) and the full analysis must therefore always be performed.

For these reasons incomplete block designs which cannot be arranged in complete replications are likely to be of less value in agriculture than ordinary lattice designs. Their greatest use is likely to be found in dealing with experimental material in which the block size is definitely determined by the nature of the material. A further use is in co-operative experiments in which each centre can only undertake a limited number of treatments. Here the use of balanced incomplete blocks (each centre forming a block) is frequently much preferable to the common practice of assigning a standard treatment (or control) to each centre.

10. SUMMARY

The recovery of inter-block information in incomplete block designs is discussed, and the method of computation is illustrated by examples.

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THE DETECTION OF LINKAGE

II. FURTHER MATING TYPES; SCORING OF BOYD'S DATA

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I. INTRODUCTION

IN a recent paper (Finney, 1940; references to the contents of this paper will be denoted by 'I') the author has given a systematic account of Fisher's method of scoring family data for linkage detection, together with such extensions of Fisher's results as were required to cover all characters dependent on only two allelomorphous genes. It is now possible to describe further extensions appropriate to some factors involving multiply allelomorphous series, the matings chosen for treatment being those arising from the *ABO* blood-group system.

Illustrations of the methods and tables now available will then be given by applying them to the data lately published by Boyd (1940). The linkages tested have already been examined by Boyd, using a test devised by Penrose (1938); it is thus possible to compare the information obtained by the two methods. Suggestions will be made for combining the two tests so as conveniently to detect linkages in large bodies of suitable data.

The notation and nomenclature of I will be used in the present paper, and will not be described again here. In particular, frequent reference will be made to the mating types enumerated in Table 1 and to the functions whose definitions are collected in Tables 22-24 of that paper.

2. THE *ABO* BLOOD GROUPS

In I, § 7 the author has made a preliminary examination of the methods of scoring appropriate to linkage tests when one factor in the test is the *ABO* blood-group classification. A more comprehensive account of the mating types involved will now be given. In order to bring this class of scoring into line with the simpler cases concerning gene pairs some modification of the previous recommendations is necessary; the present description should therefore be taken as replacing what has previously been said.*

Of the three genes to be considered here *A* and *B* are each dominant to *O* and have no relative dominance; no attempt will be made to discuss the subclassification of the *A* gene into *A*₁ and *A*₂. It is clear that in all *A* × *O*, *B* × *O*, *A* × *A*, and *B* × *B* matings the *A* or *B* gene behaves exactly as a simple dominant, and segregations may be scored in the same way as for the corresponding *T* × *t* or *T* × *T* matings shown in I, Tables 1 and 2. Similarly, *AB* × *AB* matings are scorable in the same manner as *MN* × *MN* in these tables. In *AB* × *O*

* These changes have little effect on the test of the *ABO* × Allergy linkage made in I, as the slight alteration in the scoring of families 52 and 60 scarcely influences the final figures.

matings the expected frequencies of A and B phenotypes among the children are exactly as for M and MN from $M \times MN$ parents.

The score appropriate to an $A \times B$ mating may be derived by the method of expansion exemplified in I, § 5. It is easily found that the value of this score is equivalent to that which would be obtained if the segregations of the A and B genes were scored separately; thus if both parents are heterozygous for the second factor the score is obtained in two parts. It is recommended that in such cases the appropriate values of κ should be attached to the two portions separately, an $AT \times BT$ mating being scored exactly as if it were two families, one $AT \times T$ and the other $T \times BT$.

The variance of the total score thus obtained neglects the correlation between the two portions. For the totality of families of given size this correlation is zero, but separation into 'certain' and 'doubtful' categories creates some small correlations; the magnitude of these is, however, such that they may well be ignored entirely. A simple example of this is provided by the mating $AMN \times BMN$. The resulting family proves with certainty the heterozygous nature of both parents if there is either an O child or both an A and a B . The family is doubtful for A if B and O children are both absent, doubtful for B if A and O are absent, and doubtful for both if only AB children appear. The probabilities of heterozygosity for the two parents may be denoted by p_1 and p_2 , with π_1 and π_2 for the values which p_1 , p_2 would take if all children tested had been AB . Rejecting the MN children, scores $\frac{1}{2}\rho u_{11}$ are determinable for each segregation as for type 17 of I, Table 1. Applying the usual technique of constructing a generating function and operating on it with D_{11} , it may be shown that for the certain families the variance of the total score is

$$\kappa_c = s(s-1) \left\{ 1 + \frac{1}{(2^s-1)^2} \right\}. \quad (2.1)$$

If the heterozygosity of the A parent is doubtful

$$\kappa_d = \frac{1}{2}s(s-1) \left\{ (1+p_1^2) - \frac{2\rho_1}{2^s-1} \right\}, \quad (2.2)$$

and if B is doubtful

$$\kappa_d = \frac{1}{2}s(s-1) \left\{ (1+p_2^2) - \frac{2\rho_2}{2^s-1} \right\}; \quad (2.3)$$

if both the A and the B parents are of doubtful heterozygosity

$$\kappa_d = \frac{1}{2}s(s-1) \{ (\rho_1^2 + \rho_2^2) + 2\rho_1\rho_2 \}. \quad (2.4)$$

The expected frequencies of these four classes are proportional to $(2^s-1)^2$, $(2^s-1)/\pi_1$, $(2^s-1)/\pi_2$, $1/\pi_1\pi_2$ respectively, and thus the expected variance for all families having s not- MN children is

$$\kappa = \frac{1}{2}s(s-1) \left\{ \frac{2^s-\rho_1}{2^s+\tau_1} + \frac{2^s-\rho_2}{2^s+\tau_2} \right\}, \quad (2.5)$$

where, as usual, $\rho = 1 - \pi$, $\tau = \rho/\pi$, this value of κ being obtained by combining (2.1)–(2.4) in their appropriate proportions.

This expected variance is easily seen to be the sum of the two values of ω_{15} , though the

four earlier expressions each differ slightly from the sum of the variances of the two portions of the score. In each case the last term within the bracket represents the correction for correlation; these quantities are never large in absolute magnitude, and as their expected value for all families of size s is zero the effect of ignoring them entirely cannot be of much importance. The same procedure, leading to similar results, could be followed with other $A \times B$ matings, in all cases the separate scoring of the two segregations being the practical solution of the difficulty. Of course if only one parent is heterozygous for the second factor, only the one segregation is scorable and this issue does not arise.

The reason underlying this peculiarity is that, for families of the mating discussed whose only scorable children are AB , the values of u_{11} are the same for both segregations and the two portions of the score are completely correlated. The variance of the total score, $\lambda = \frac{1}{2}(p_1 + p_2)u_{11}$, is then clearly $(p_1 + p_2)^2 \omega_1$ and not $(p_1^2 + p_2^2) \omega_1$. For the other three classes of family of given size the introduction into the generating function of a term representing the rejection of families having only AB children causes compensating correlations. The ignoring of these small corrections to the empirical variances bears some analogy to the use of κ_0 , an additional quantity of information for distribution of families of given size into subgroups, which was discussed in I, § 6. It should be noted that here, as everywhere, the class to which a family belongs (certain for both parents, certain for one and doubtful for the other, or doubtful for both) is determined from the scorable children only. Nevertheless, it is possible that the phenotype of a rejected MN child may show that the heterozygosity probability, which must be used in the scoring of a so-called doubtful segregation, is unity.

This last point was perhaps not made sufficiently clear in I. It is important to notice that the decision as to whether a family shall be scored from Table 1 or from Table 2 of that paper is to be based only on the scorable children, though the value of p to be used in Table 2 may possibly be derived from a number which includes unscorable sibs. For example, an $AMN \times AMN$ mating having two AM children is of type 19, with score $\frac{2}{3}p$ and variance $\frac{4}{9}p^2$ obtained as in I, Table 2. The value of p , the probability that both parents are heterozygous, is, for a western European population, 0.5611 (from I, Table 25). Had there also been an AMN child, this would not be available for scoring, but would change the value of p to 0.4895. Alternatively, had there been an OMN child, the family is still classed as 'doubtful', though the value of p now becomes unity; the variance is therefore $\frac{4}{9}$ and not 0.9524 as would be the case with a 'certain' family of size 2. The same rules apply to the more complex matings of the present paper.

The remaining types, $A \times AB$ and $B \times AB$, are more troublesome; as these are symmetrical, only the former need be analysed. Again the score consists of two parts; the first represents the segregation of B and is formed from all phenotypes (except, of course, MN in $AMN \times ABMN$), and the second represents the segregation of A for B and AB children only, since this segregation cannot be observed in the A children. Again if only one parent is doubly heterozygous, only the corresponding segregation is scorable. In

Tables 1 and 2 these scores are catalogued, together with the appropriate values of κ , whose derivation is now to be described.

It will be recalled that in $MN \times MN$ matings MN children are not used in the formation of λ . Two methods might be adopted in finding the variance of λ , since a family may be considered as a member of the population of families having a given total number of children or as a member of the population of families having a given number of not- MN children. The latter method has in general been used in I, Tables 1 and 2, since the results are somewhat simpler and on theoretical grounds slightly to be preferred as making use of the ancillary information provided by the number of not- MN children. The difference between the two values of κ may be illustrated from type 21 ($MNP \times MNPQ$); I, Table 1 gives the value as $\frac{1}{2}s(s-1)$, whereas the alternative method gives $\frac{1}{8}s'(s'-1)$, s being the

Table 1. *Table of scoring systems for ABO matings*

Type	Mating	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	λ	λ	κ_c
1'	$ABd \times AD$	ABD	BD	ABd	Bd	$\frac{1}{2}u_{11}$	—	ω_1
3'	$Ad \times ABD$	AD	BD, ABD	Ad	Bd, ABd	$\frac{1}{2}u_{11}$	—	ω_1
5'	$ABr \times AR$	ABR	BR	ABr	Br	$\frac{1}{2}u_{11}$	—	ω_1^*
9'	$Ar \times ABR$	AR	BR, ABR	Ar	Br, ABr	$\frac{1}{2}u_{11}$	e_8	ω_8
11'	$ABR \times AR$	(1) AR	Ar	BR, ABR	Br, ABr	$\frac{1}{2}u_{31}$	—	ω_1
7'	$ABR \times AR$	(2) ABR	ABr	BR	Br	$\frac{1}{2}u_{31}$	—	ω_3
						$\frac{1}{2}u_{31}$	e_{10}	ω_{10}^*
13'	$ABt \times AT$	ABT	BT	ABt	Bt	$\frac{1}{2}u_{11}$	e_5	ω_5
16'	$At \times ABT$	AT	BT, ABT	At	Bt, ABt	$\frac{1}{2}u_{11}$	—	ω_1
18'	$ABT \times AT$	(1) AT	At	BT, ABT	Bt, ABt	$\frac{1}{2}u_{31}$	—	ω_2
14'	$ABT \times AT$	(2) ABT	ABt	BT	Bt	$\frac{1}{2}u_{31}$	e_6	ω_6
16'	$ABM \times AMN$	$ABMN$	BMN	ABM	BM	$\frac{1}{2}u_{11}$	—	ω_1
20'	$AM \times ABMN$	AMN	$BMN, ABMN$	AM	BM, ABM	$\frac{1}{2}u_{11}$	—	ω_1
21'	$ABMN \times AMN$ (1)	AM	AN	BM, ABM	BN, ABN	$\frac{1}{2}u_{11}$	—	ω_1
21'	$ABMN \times AMN$ (2)	ABM	ABN	BM	BN	$\frac{1}{2}u_{11}$	—	ω_1

* For these κ values, s is to be taken as the total number of children inclusive of A .

number of M and N children and s' the total number of children. In practice this difference and those of a similar kind in other matings are unimportant, and a convention that the 'reduced' value of the family size shall be used avoids the necessity of having some additional tables. Exceptions to this rule are types 10 and 12 for known ascertainment, these types concerning recessive abnormalities; here ascertainment may be made through recessive children whether or not they are MN and the values of κ are therefore calculated for the 'unreduced' family size. Even for these types, if ascertainment procedure is not known, reduced family size is to be used for the κ values in the (s_1, s_2) scoring system.

A similar situation arises with the A segregation of an $A \times AB$ mating. Though A children are not used in the formation of this portion of the score, it would be possible to find the variance of that score in all families of given total size. There is, however, again some

theoretical preference for the alternative of finding the variance in families having a given number of AB and B children; this approach also has the not inconsiderable advantage of avoiding the introduction of a further long series of tables. Instead, it is found that, for example, the expected relative frequencies of ABD , BD , ABd and Bd children from a $ABd \times AD$ mating are exactly the same as those of TD , tD , Td and td children from a $Td \times TD$ mating. Most of the results shown in Tables 1 and 2 are thus immediately obtained from I, Tables 1 and 2, type 1' corresponding to type 1 as just described. All segregations of B are immediately identifiable with the corresponding undashed segregations of I. As has been mentioned above, when both parents are doubly heterozygous the two segregations are to be scored separately in the manner shown in the table, the effects of correlation of the two parts introduced by division into certain and doubtful families once more being negligible and of zero expectation.

Table 2. Table of scoring systems for ABO matings (inclusion of 'doubtful' families)

Type	Mating	λ	λ	κ_d	κ_0	κ_e
1'	$ABd \times AD$	$\frac{1}{2}pu_{11}$	—	$p^2\omega_1$	—	ω_{15}
5'	$ABr \times AR$	$\frac{1}{2}pu_{11}$	—	$\frac{1}{2}p^2\omega_1^*$	—	ω_{15}^*
			—	—	$\pi\gamma_8$	ω_{22}
7'	$ABR \times AR$ (2)	$\frac{1}{2}pu_{31}$	—	$\frac{1}{2}p^2\omega_3^*$	—	ω_{17}^*
			—	—	$\pi\gamma_{10}$	ω_{24}
13' ($b=d=o$)	$ABt \times AT$	$\frac{1}{2}p_0u_{11}$	p_0e_{13}	$p_0^2\omega_{13}$	$\pi_0\pi_t\gamma_5$	ω_{19}
13' ($c=d=o$)	$ABt \times AT$	$\frac{1}{2}p_tu_{11}$	p_te_{13}	$p_t^2\omega_{13}$	$\pi_0\pi_t\gamma_5$	ω_{19}
16' ($c=d=o$)	$At \times ABT$	$\frac{1}{2}p_tu_{11}$	—	$p_t^2\omega_1$	—	ω_{15}
18' ($b=d=o$)	$ABT \times AT$ (1)	$\frac{1}{2}p_tu_{31}$	—	$s_1^2p_t^2\omega_1$	—	ω_{16}
14' ($b=d=o$)	$ABT \times AT$ (2)	$\frac{1}{2}p_te_{31}$	$\frac{1}{2}p_te_{13}$	$s_1^2p_t^2\omega_{13}$	$\pi_0\pi_t\gamma_6$	ω_{20}
14' ($c=d=o$)	$ABT \times AT$ (2)	$\frac{1}{2}p_0u_{31}$	p_0e_{14}	$p_0^2\omega_{14}$	$\pi_0\pi_t\gamma_6$	ω_{20}
16'	$ABM \times AMN$	$\frac{1}{2}pu_{11}$	—	$p^2\omega_1$	—	ω_{15}

Where two probabilities of heterozygosity have to be considered, p_0 , π_0 refer to the heterozygosity of A , p_t , π_t to that of T .

* For these κ values, s is to be taken as the total number of children inclusive of A .

Once again there is a complication with recessive abnormalities, since again allowance must be made for the possibility of ascertaining a family through a recessive whose phenotype is not used in the formation of the score. This remark applies to $ABr \times AR$ and $ABR \times AR$, but not to $Ar \times ABR$ for which the B segregation is scorable on all children. The expectations of the six phenotypes in a family of size s from an $ABR \times AR$ mating in the absence of linkage are:

Phenotype	ABR	ABr	BR	Br	AR	Ar
Expected frequency	$\frac{3}{16}s$	$\frac{1}{16}s$	$\frac{3}{16}s$	$\frac{1}{16}s$	$\frac{3}{8}s$	$\frac{1}{8}s$
Observed frequency	a	b	c	d	e	f

The scoring of the B segregation for all children presents no difficulty and the instructions are given in Table 1. For the A segregation on the 'certain' families (i.e. those having at least one B child which ensures the heterozygosity of the A parent), the probabilities of ascertainment of families of the possible constitutions are proportional to the coefficients of terms $\alpha^a \beta^b \gamma^c \delta^d \theta^{e+f}$ in the expansion of the generating function

$$\Phi = s(\beta + \delta + 2\theta)(3\alpha + \beta + 3\gamma + \delta + 8\theta)^{s-1} - s(\beta + 2\theta)(3\alpha + \beta + 8\theta)^{s-1}, \quad (2.6)$$

the distinction between AR and Ar now being dropped since neither is used for the score. This generating function is derived as in I, § 6. In that paper the values of κ were always found as the multiples of $(1-4\xi)$ in the expectation of the score, assuming there to be a frequency, χ , of crossing-over; it was, however, proved that the same functions could be derived as the variance of the score in the absence of linkage, and the possibility of that approach will be illustrated here, where it has certain manipulative advantages.

Putting $\alpha = \beta = \gamma = \delta = \theta = 1$, the total frequency is

$$\Sigma f = s4^{s-1}(4^s - 3^s). \quad (2.7)$$

Application of the operator D_{31} shows that

$$\begin{aligned} D_{31}\Phi &= s(s-1)(s-2)(\beta + \delta + 2\theta)(3\alpha - 3\beta - 3\gamma + 3\delta)(3\alpha + \beta + 3\gamma + \delta + 8\theta)^{s-3} \\ &\quad + 2s(s-1)(-3\beta + 3\delta)(3\alpha - 3\beta - 3\gamma + 3\delta)(3\alpha + \beta + 3\gamma + \delta + 8\theta)^{s-2} \\ &\quad - s(s-1)(s-2)(\beta + 2\theta)(3\alpha - 3\beta)^2(3\alpha + \beta + 8\theta)^{s-3} \\ &\quad - 2s(s-1)(-3\beta)(3\alpha - 3\beta)(3\alpha + \beta + 8\theta)^{s-2}, \end{aligned} \quad (2.8)$$

and a second application followed by writing $\alpha = \beta = \gamma = \delta = \theta = 1$ shows that

$$\Sigma f u_{31}^2 = 18s(s-1)(s+4)4^{s-1}(4^{s-1} - 3^{s-2}). \quad (2.9)$$

Similarly for the doubtful families (i.e. those having no B child), the generating function

$$\Phi = s(\beta + 2\theta)(3\alpha + \beta + 8\theta)^{s-1}/\pi \quad (2.10)$$

leads to

$$\Sigma f = s3^s 4^{s-1}/\pi, \quad (2.11)$$

and

$$\Sigma f p^2 u_{31}^2 = 2\pi 3^s 4^{s-1} s(s-1)(s+4). \quad (2.12)$$

Combining certain and doubtful families, from (2.7) and (2.11)

$$\Sigma f = s4^{s-1}(4^s + 3^s \tau), \quad (2.13)$$

and from (2.9) and (2.12)

$$\Sigma f p^2 u_{31}^2 = 18s(s-1)(s+4)4^{s-1}(4^{s-1} - 3^{s-2} \rho). \quad (2.14)$$

Now the score to be used is

$$\lambda = \frac{1}{18} p u_{31}. \quad (2.15)$$

Hence

$$\kappa = \frac{1}{18}(s-1)(s+4) \frac{4^{s-1} - 3^{s-2} \rho}{4^s + 3^s \tau} = \omega'_{17}, \quad \text{say.} \quad (2.16)$$

For the certain families alone

$$\kappa_c = \frac{1}{18}(s-1)(s+4) \frac{4^{s-1} - 3^{s-2}}{4^s - 3^s} = \omega'_3, \quad (2.17)$$

and for the doubtful families alone

$$\kappa_d = \frac{1}{162} p^2 (s-1)(s+4) = \frac{1}{9} p^2 \omega_3. \quad (2.18)$$

It may be observed that I, (7.2)–(7.4) correspond to these last three equations with $s = 3$. In the case of unknown ascertainment procedure, when the (s_1, s_2) scoring system is to be used, there is no necessity to make any use of the number of A children, and the ordinary type 7 method should be used on the reduced family size rather than the method indicated in I, § 7.

For an $ABr \times AR$ mating similar treatment shows that for all families

$$\kappa = \frac{1}{2}s(s-1) \frac{4^{s-1} - 3^{s-2}\rho}{4^s + 3^s\tau} = \omega'_{15}, \quad (2.19)$$

and for certain and doubtful families separately

$$\kappa_c = \frac{1}{2}s(s-1) \frac{4^{s-1} - 3^{s-2}}{4^s - 3^s} = \omega'_1, \quad (2.20)$$

$$\kappa_d = \frac{1}{8}p^2s(s-1) = \frac{1}{8}p^2\omega_1. \quad (2.21)$$

These new functions (ω'_1 and ω'_3) are tabulated in Table 3. Together with the tables of I, this completes the set of tables required for the testing of linkages with the ABO system.

Table 3. *Table of ω'_1 and ω'_3*

s	ω'_1	ω'_3
2	0.428 57	0.142 86
3	1.054 05	0.273 27
4	1.885 71	0.419 05
5	2.932 14	0.586 43
6	4.201 07	0.777 98
7	5.690 30	0.995 12
8	7.432 64	1.238 77
9	9.405 90	1.509 59
10	11.622 06	1.808 02
11	14.086 86	2.134 37
12	16.799 87	2.488 87
13	19.763 03	2.871 64
14	22.979 28	3.282 75
15	26.447 52	3.722 24
16	30.168 73	4.190 10

$$\omega'_1 = \frac{1}{2}s(s-1) \frac{4^{s-1} - 3^{s-2}}{4^s - 3^s}, \quad \omega'_3 = \frac{1}{8}(s-1)(s+4) \frac{4^{s-1} - 3^{s-2}}{4^s - 3^s}.$$

3. BOYD'S DATA AND PENROSE'S SIB METHOD

Boyd has recently published (1940) data concerning 202 families from various Near Eastern populations. He has recorded, for each subject, sex, ABO and MN blood groups, ability to taste phenyl-thiocarbamide, presence of hair in the mid-digital region of the fingers, and degree of pigmentation of hair, eyes and skin. For thirty of the families both parents were examined, for thirty-four one parent only was examined, and for the remaining 138 neither parent was examined. The linkages of these eight characters were tested by the method of sib-pairs, developed by Penrose (1938) for graded human characters. The records

of mid-digital hair and pigmentation grades will not be used here, since for the first there is no clear case for the dominance of 'hair present' and for the others no genetic model is propounded.

The method of Penrose* consists of taking from each family every possible pair of children and scoring their degree of difference in each character as 0 or 1 for factors showing dominance, 0, 1 or 2 for factors without dominance. If these differences are d_1 , d_2 , the measure of linkage from n sib pairs is

$$\phi = \frac{nS(d_1^2 d_2^2)}{S(d_1^2) S(d_2^2)} - 1, \quad (3.1)$$

the variance of which is
$$V = \frac{(n^2 S(d_1^4) S(d_2^4) - 1)}{S^2(d_1^2) S^2(d_2^2)} \bigg/ (n-1). \quad (3.2)$$

From ϕ an estimate of the recombination fraction may be obtained. For two factors without dominance this is given by

$$1 - 4x = 2\phi; \quad (3.3)$$

if one factor is recessive, the population frequency of the dominant gene being ν_1 , the equation of estimation is

$$1 - 4x = \frac{3 + \nu_1}{1 + \nu_1} \phi; \quad (3.4)$$

if neither factor is recessive, the frequencies of the dominant genes being ν_1 , ν_2 , the equation becomes

$$1 - 4x = \frac{9 + 3(\nu_1 + \nu_2) + \nu_1 \nu_2}{3 + (\nu_1 + \nu_2) + 3\nu_1 \nu_2} \phi. \quad (3.5)$$

If the equation of estimation is $1 - 4x = \eta\phi$, (3.6)

it is clear that the standard error of $(1 - 4x)$ is

$$\sigma = \eta V^{\frac{1}{2}}, \quad (3.7)$$

and the information obtained about $(1 - 4\xi)$ is $1/\sigma^2$.

The results obtained by Boyd have been transformed to the standard estimation of $(1 - 4\xi)$ from these equations. For this purpose the frequencies of the A , B and T genes have been taken as $\frac{4}{15}$, $\frac{1}{15}$ and $\frac{1}{2}$ respectively. These frequencies are based on a Western European population; in Boyd's data the frequency of B is, as might be expected, rather higher, but, as (3.4) and (3.5) are not very sensitive to small changes in ν , it was not considered necessary to revise these estimates. The correct estimation of $(1 - 4\xi)$ for multiple allelomorphs has not been considered by Penrose, and Boyd has scored the linkages of A and B separately—or, more often, scored A only—but it may reasonably be assumed that the formulae will remain substantially correct. In Table 4 are given the results of transforming the values of ϕ from Boyd's Table B.

* The notation adopted by Penrose has been changed slightly so as to agree more closely with that introduced in I.

This method of linkage detection entirely ignores any information given by the parents of the sibs. The use of those families which have no doubly heterozygous parent, and therefore can show no crossing-over, should only introduce an additional random element into the score, but there may be a considerable loss of information through scoring other families by a method not fully efficient. This possibility is discussed in more detail in the following sections.

The data collected by Boyd are from a number of different racial groups. It is possible that any marked heterogeneity of gene frequencies between the different populations might lead to a spurious indication of linkage when the whole sample is scored at the same time. This effect could not occur with the system of scoring based on u -statistics, since each family provides an additive component to the total score. The use of an incorrect value for a gene frequency in that case would only result in a measure of faulty weighting of the scores of the doubtful families, which in any event contribute little to the total. A further weakness of the method of sib-pairs is that it appears to attach undue weight to the large families by scoring separately every possible pair of children.

Table 4. *Estimates of linkage and quantities of information (Boyd's data)*

Linkage	$(1-4x)$	σ	Information
Sex \times A	0.227	0.317	9.950
Sex \times B	-0.492	0.489	4.186
Sex \times MN*	0.129	0.260	14.781
Sex \times T	0.490	0.411	5.917
A \times MN	0.444	0.379	6.958
A \times T	0.475	0.530	3.565
MN \times T	-0.940	0.485	4.245

* This linkage was not tested by Boyd; 503 pairs of sibs have been scored from his data by the present author.

In the six sections which follow the linkages of sex, *ABO* and *MN* types, and taste test reaction will be re-examined by efficient scoring of those families for which the parental phenotypes have been recorded; in every case a considerable increase in information is obtained. A further section will suggest how best the estimates from these families and from the remainder may be combined.

4. SEX \times ABO

Of the thirty families for which both parents are recorded, twenty-three provide information on the possibility of linkage of sex and the *ABO* system. Table 5 shows the scoring of these families, set out in a manner similar to that of the linkages scored in I.

The first column contains the family number in Boyd's data. Examination of the parental and child phenotypes enables the next five columns to be filled according to the schemes of Table 1 of I or of the present paper. Next is shown which of the genes *A*, *B* is segregating; for an *AB* \times *O* or *AB* \times *AB* mating this column is left empty. The total number of children scored is shown under the head of s . In the next column is given the probability of heterozygosity of the father; this is obtained from I, Table 25 if the heterozygosity is not proved

by the children, the value of s to be used in this table possibly being larger, since there may be children giving information on this point but not available for the score. The probabilities of I, Table 25 were based on gene frequencies in a western European population, and consequently the frequency of the B gene used there is likely to be lower than that in the races studied by Boyd. The observed frequencies of the different phenotypes support this view, but in all linkages studied the total information contributed by the doubtful families is so small as to make it scarcely worth while to use corrected gene frequencies. The value of λ/p is computed, by use of the appropriate u -statistic, and multiplied by p , the corresponding value of κ being obtained from the tables of the appropriate ω function. Acting

Table 5. *Scoring of Sex \times ABO*

Family	Type	a	b	c	d	Segre- gating	s	p	λ/p	λ	κ
119	18	2	—	2	1	A	5	1.0000	-0.2222	-0.222	1.418
88	16	—	2	—	—	A	2	1.0000	1.0000	1.000	1.000
89	20	2	—	—	—	—	2	1.0000	1.0000	1.000	1.000
132	16'	1	1	1	1	B	4	1.0000	-2.0000	-2.000	6.000
1	18	4	—	4	—	B	8	0.4941	-0.4444	-0.220	0.084
3	16	2	1	—	1	B	4	1.0000	0	0	6.000
65	16'	—	2	—	—	B	2	1.0000	1.0000	1.000	1.000
147	18	1	—	2	—	A	3	0.4895	-0.1111	-0.054	0.009
139	16'	—	—	2	—	A	2	1.0000	1.0000	1.000	1.000
140	20	1	1	1	2	—	5	1.0000	-2.0000	-2.000	10.000
14 ^b	18	2	2	—	—	B	4	1.0000	-0.2222	-0.222	0.941
14 ^c	18	1	—	1	—	B	2	0.8459	-0.1111	-0.094	0.009
15	18	1	1	2	—	A	4	1.0000	0.2222	0.222	0.941
12	16	—	1	1	1	A	3	1.0000	-1.0000	-1.000	3.000
13	16	1	—	3	—	B	4	0.5556	0	0	1.852
16	16	—	3	2	2	A	7	1.0000	1.0000	1.000	21.000
11	20'	4	—	1	—	B	5	1.0000	2.0000	2.000	10.000
129	16'	—	—	1	1	B	2	1.0000	-1.0000	-1.000	1.000
130	20'	—	2	—	—	B	2	1.0000	1.0000	1.000	1.000
149	18	1	1	2	1	A	5	1.0000	-1.1111	-1.111	1.418
150	16	1	—	1	—	A	2	0.5556	-1.0000	-0.556	0.309
123	16	1	—	1	—	B	2	0.8333	-1.0000	-0.833	0.694
144	16	—	—	2	1	A	3	1.0000	-1.0000	-1.000	3.000
Total							82			-2.090	72.675

on the recommendation of I, only the observed quantities of information have been computed, the values of κ_e being ignored.

Thus family 65 is a mating $\delta B \times \phi AB$ having two δB and two δA children. The existence of A children proves the father to be heterozygous for B and therefore p is unity. The segregation of B is exactly as for a mating of $ABM \times BMN$ and is thus scorable as type 16' of Table 1, the B children being rejected and the δA children falling into class b . The score to be used is $\frac{1}{2}u_{11}$, which is 1.000, and the value of κ is $\frac{1}{2}s(s-1)$, which is also 1.000, since s , the number of children used in the score, is two.

Again, family 147 is $\delta A \times \phi A$ with one ϕA and two δA children. This is immediately seen to be of type 18 (I, Table 1), ϕA being classified as a and δA as c . Since there is no

O child, the probability that the parents should be heterozygous for A is obtained from the fourth column of I, Table 25 with $s = 3$; the value of p is 0.4895. Now $\frac{1}{18}u_{31}$ is $-1/9$, and this multiplied by p gives the score as -0.054 . From I, Table 2, the value of κ is $\frac{1}{81}p^2\omega_1$, which, since ω_1 is 3 for $s = 3$, is found to be 0.009.

The total score from all families is

$$S(\lambda) = -2.090, \quad (4.1)$$

and the information on $(1 - 4\xi)$ is $S(\kappa) = 72.675. \quad (4.2)$

The estimate, $(1 - 4x)$, is obtained by division, its variance being $1/s(\kappa)$, and is

$$1 - 4x = -0.029 \pm 0.117. \quad (4.3)$$

Now the total information on both $\text{Sex} \times A$ and $\text{Sex} \times B$ linkages obtained by the Penrose method was (Table 4) only 14.136 from all families. Efficient scoring methods have extracted five times as much information from thirty families only. Efficient scoring of the remaining families would increase this information still further, but of course not in the same proportion, since one or both parents are missing from the records. As there is not the slightest suggestion of linkage there would be little practical interest in extending this scoring to the incomplete material; from the theoretical point of view it is desirable that the technique should be developed for these families, but, except in a few simple cases (see § 5), the analysis required is likely to prove very laborious.

5. $\text{SEX} \times MN$

By the methods of I this pair of factors may be scored for linkage in all families of which the father is MN . There are eleven such families among the thirty for which both parents are recorded, and the details of the scoring are shown in Table 6.

Table 6. *Scoring of $\text{Sex} \times MN$*

Family	Type	a	b	c	d	s	λ	κ
119	21	1	2	—	—	3	-1	3
120	20	1	—	1	—	2	-1	1
88	21	—	—	2	—	2	1	1
132	20	—	2	2	—	4	6	6
1	21	—	2	—	—	2	1	1
65	20	3	1	—	—	4	0	6
148	20	2	—	—	—	2	1	1
13	21	1	1	—	1	3	-1	3
130	20	1	1	—	—	2	-1	1
149	20	2	1	2	—	5	-2	10
150	20	—	1	1	—	2	1	1
Total						31	4	34

The scoring of these families is particularly simple and needs no further comment. With the mating types occurring here it is also possible to make effective use of some of those families for which only one parent is recorded. (In this connexion see Fisher (1935).)

Consider the mating $\delta ? \times \varphi M$; this will provide information on linkage if, and only if, the father is MN , a condition of which the probability may be assessed from the numbers of M and MN children. The expected frequencies in a family of size s from a $\delta MN \times \varphi MN$ mating are, in the absence of linkage:

Phenotype	φM	φMN	δM	δMN
Expected frequency	$\frac{1}{2}s$	$\frac{1}{2}s$	$\frac{1}{2}s$	$\frac{1}{2}s$
Observed frequency	a	b	c	d

If both M and MN children are present, the father must be MN and the generating function is

$$\Phi = (\alpha + \beta + \gamma + \delta)^s - (\alpha + \gamma)^s - (\beta + \delta)^s, \quad (5.1)$$

the total frequency of such families, obtained by putting $\alpha = \beta = \gamma = \delta = 1$, being

$$\Sigma f = 2^s(2^s - 2). \quad (5.2)$$

The usual argument shows the efficient score to be

$$\lambda = \frac{1}{2}u_{11}, \quad (5.3)$$

and thus the operator D_{11} is required. Operating twice on Φ and writing $\alpha = \beta = \gamma = \delta = 1$, it is found that

$$\Sigma f u_{11}^2 = 2^{s+1}s(s-1)(2^s - 2). \quad (5.4)$$

Hence

$$E(\lambda^2) = \frac{1}{2}s(s-1) = \omega_1 \quad (5.5)$$

is the value of κ_c for these families.

If there are only M children it follows that the father must be M or MN . Suppose ν_1 to be the probability that a random individual known to be M or MN should be MN ; then the probability that the father of this family is MN is

$$p_1 = \frac{\nu_1}{\nu_1 + 2^s(1 - \nu_1)}. \quad (5.6)$$

As in I, § 4 it is necessary to introduce the function π_1 the value of p_1 if all children tested in a family of size s had been found to be M . Then for families of M children only the generating function is

$$\Phi = (\alpha + \gamma)^s / \pi_1, \quad (5.7)$$

the total frequency is

$$\Sigma f = 2^s / \pi_1, \quad (5.8)$$

and

$$\Sigma f u_{11}^2 = 2^{s+1}s(s-1) / \pi_1. \quad (5.9)$$

The score for these families is

$$\lambda = \frac{1}{2}p_1 u_{11}, \quad (5.10)$$

and the variance

$$\kappa_d = p_1^2 \omega_1. \quad (5.11)$$

Again, if only MN children are present, the father might be MN or N . Defining ν_2 as the probability that a random individual known to be MN or N should be MN ,

$$p_2 = \frac{\nu_2}{\nu_2 + 2^s(1 - \nu_2)} \quad (5.12)$$

is the probability that the father should be MN . For families of MN only, the score is

$$\lambda = \frac{1}{2}p_2 u_{11}, \quad (5.13)$$

with

$$\kappa_d = p_2^2 \omega_1. \quad (5.14)$$

Finally, taking all families together, the scores to be used are as above, and the variance, information, and score divisor has the expected value

$$\kappa_e = \frac{1}{2}s(s-1) \frac{2^s - \rho_1 - \rho_2}{2^s + \tau_1 + \tau_2}, \quad (5.15)$$

where

$$\rho = 1 - \pi, \quad \tau = \rho/\pi. \quad (5.16)$$

This mating will be referred to as type 20.1.

A similar analysis may be made for the mating ♂ ? × ♀ *MN*, type 21.1. Here the heterozygosity of the father can only be proved by the presence of both *M* and *N* children. The score is then

$$\lambda = \frac{1}{2}u_{11}, \quad (5.17)$$

with

$$\kappa_e = \omega_1, \quad (5.18)$$

the value of *s* being the family size after rejection of any *MN* children. If *N* children are absent, the probability of heterozygosity is (ν_1 as defined above)

$$p_1 = \frac{3^s \nu_1}{3^s \nu_1 + 4^s (1 - \nu_1)}, \quad (5.19)$$

using the total number of children recorded to determine p_1 . For such families the score is

$$\lambda = \frac{1}{2}p_1 u_{11}, \quad (5.20)$$

and

$$\kappa_d = p_1^2 \omega_1. \quad (5.21)$$

For families lacking *M* children, p_1 is replaced by p_2 , where

$$p_2 = \frac{3^s \nu_2}{3^s \nu_2 + 4^s (1 - \nu_2)}. \quad (5.22)$$

Once more, for all families
$$\kappa_e = \frac{1}{2}s(s-1) \frac{2^s - \rho_1 - \rho_2}{2^s + \tau_1 + \tau_2}, \quad (5.23)$$

s here being the reduced family size.

The remaining mating of this group is ♂ *MN* × ♀ ?; greater difficulties are encountered in the analysis of this, since it provides information on linkage whatever the genotype of the mother, and one family may possess two components of score corresponding to two possible genotypes. For the present no further attempt will be made to provide an efficient score for these families of which the mother is unrecorded.

For the data under examination the frequencies of the *M* and *N* genes have been taken to be equal. It is likely that the *M* gene is actually the more common, but the difference is small, and, as with other cases of doubtful families, the total information to be obtained from families whose paternal genotype is uncertain is so small as to be little influenced by slight changes in weighting. This choice of gene ratio enables probabilities of heterozygosity for type 20.1 to be obtained directly from the first column of I, Table 25; for type 21.1 they must be computed from (5.19).

Of the thirty-four families for which one parent is unrecorded, twenty-three lack the paternal record and fourteen of these contribute to the linkage score. The remainder are

useless because of rejection of all or all but one child in type 21.1. These fourteen families are scored in Table 7.

For example, family 155 has parents $\delta ? \times \varphi MN$, the three children being δMN , φN and δN . This is clearly of type 21.1, so that the MN child must be rejected and the others placed in classes d and b respectively. Of three children, none are M , and the probability that the father is in reality MN is therefore given by (5.22) with $s = 3$. For equal gene frequencies $\nu_2 = \frac{2}{3}$ and this probability is found to be 0.2195. Now $\frac{1}{2}u_{11} = -1$, and the score, λ , is thus -0.220 . The value of κ is $\frac{1}{2}p^2s(s-1)$ for $s = 2$, or 0.048. So the total score and total information are obtained, it being noticeable that 90% of the latter is obtained from the certain families.

Table 7. *Scoring of Sex \times MN for incomplete families*

Family	Type	a	b	c	d	s	p	λ/p	λ	κ
166	20.1	---	---	2	---	2	0.3333	1	0.333	0.111
58	20.1	---	---	1	1	2	1.0000	-1	-1.000	1.000
152	20.1	2	---	---	---	2	0.3333	1	0.333	0.111
96	20.1	---	---	1	1	2	1.0000	-1	-1.000	1.000
155	21.1	---	1	---	1	2	0.2195	-1	-0.220	0.048
160	20.1	---	2	---	1	3	0.2000	-1	-0.200	0.120
161	20.1	---	---	2	---	2	0.3333	1	0.333	0.111
163	20.1	1	---	---	1	2	1.0000	1	1.000	1.000
124	20.1	---	---	2	---	2	0.3333	1	0.333	0.111
141	21.1	---	---	1	1	2	1.0000	-1	-1.000	1.000
142	21.1	2	---	---	---	2	0.1742	1	0.174	0.030
159	20.1	1	---	1	---	2	0.3333	-1	-0.333	0.111
94	21.1	2	---	---	1	3	1.0000	3	3.000	3.000
153	21.1	---	1	---	2	3	0.1742	-1	-0.174	0.091
Total						31			1.579	7.844

Taking Tables 6 and 7 together, $S(\lambda) = 5.579$, (5.24)

the total information being $S(\kappa) = 41.844$. (5.25)

As before, an estimate of $(1 - 4\xi)$ is obtained as

$$1 - 4x = 0.133 \pm 0.155. \quad (5.26)$$

The increase in information as compared with the Penrose method is not so marked here as with the Sex \times ABO test. Nevertheless, the information from the first thirty families alone is more than twice the total from all families obtained by sib-pairs. It is of interest to examine this gain in greater detail. The 503 sib-pairs from all families may be divided into four sets: (i) 160 from 29 families with both parents recorded for MN type, (ii) 43 from 23 families with father not recorded, (iii) 43 from 11 families with mother not recorded, (iv) 257 from 137 families with neither parent recorded. For each of these sets the Penrose score may be computed separately and an estimate of $(1 - 4\xi)$ obtained, together with its information content. The first two of these components are then directly comparable with efficient estimates from the same families given by Tables 6 and 7. The results are summarized in Table 8.

The last line of this table is obtained by forming a mean of the estimates, $(1-4x)$, weighted according to the corresponding quantities of information. On account of the non-additive nature of the Penrose scores, the estimate obtained from them in Table 8 is a little different from that of Table 4, and the information is a little greater. This table shows that, when both parents are known, scoring by sib-pairs and the consequent ignoring of the parents results in a loss of four-fifths of the information available. From those families in which only the mother is known two-thirds of the information is similarly lost. It seems reasonable to suppose that, when scored by sib-pairs, families of set (iii) lose about the same proportion of the available information as do those of set (ii). Presumably also families having neither parent recorded will lose a considerably smaller proportion of information on account of inefficiency of scoring.

Table 8. *Comparison of two tests for Sex \times MN*

Set of families	Penrose		<i>u</i> -statistics	
	$(1-4x)$	Information	$(1-4x)$	Information
(i)	0.161	6.340	0.118	34.000
(ii)	0.426	2.444	0.201	7.844
(iii)	0.112	2.145	—	—
(iv)	-0.014	4.878	—	—
All	0.141	15.807	0.133	41.844

In any collection of data such as that of Boyd, it seems likely that by far the larger part of the available information on a linkage will be provided by those families for which both parents have been examined for both factors, even though such families form a not very large proportion of the data. It is clearly important that these should be scored with maximum efficiency. If one parent was not observed for either factor the loss of information due to inefficient scoring may not be unduly large, since the total available may itself be much less than that obtained from complete families. For a sex linkage, such as is considered above, the loss of a parent is, of course, a less serious matter, since the genotype is only in doubt for one factor, and in such a case a more efficient treatment of the data may repay the labour involved. Finally, if neither parent was examined, the experience of the single linkage studied in this section would suggest, though the evidence is admittedly slight, that the loss of information by use of the sib-pair method will not be serious even for a sex linkage,* and may well be ignored unless a very refined test is required. Such considerations would clearly become more important if the data consisted entirely or almost entirely of sibships of unspecified parentage. The scores obtained by these different methods may be combined as will be shown in § 10.

* See note at foot of p. 29.

6. $\text{Sex} \times T$

The scoring of this linkage test does not require any discussion or tables beyond what has been given in I, the only possible matings when both parents are recorded being types 16 and 18. The details are shown in Table 9.

As an example, family 65 is a mating of $\delta T \times \text{female } T$ having three δT children, the remaining child not having his taste reaction recorded. The mating is clearly type 18 and of doubtful heterozygosity, since there are no t children. The children fall into class c of the scheme of I, Table 1. The probability that the parents should be heterozygous is obtained from I, Table 25 and is 0.2523. As the value of $\frac{1}{18}u_{31}$ is $\frac{1}{3}$, the score is 0.084. The variance is $\frac{1}{81}p^2\omega_1$ for $s = 3$, the value of which is 0.002.

Table 9. *Scoring of Sex $\times T$*

Family	Type	a	b	c	d	s	p	λ/p	λ	κ
120	18	1	—	—	1	2	1.0000	0.3333	0.333	0.238
132	16	1	1	2	—	4	1.0000	0	0	0.000
65	18	—	—	3	—	3	0.2523	0.3333	0.084	0.002
147	16	—	1	2	—	3	1.0000	3.0000	3.000	3.000
148	18	—	—	2	—	2	0.3103	0.1111	0.034	0.001
140	18	—	2	—	2	4	1.0000	-2.0000	-2.000	0.941
15	18	2	—	—	—	2	0.3103	0.1111	0.034	0.001
12	18	—	—	1	1	2	1.0000	-0.3333	-0.333	0.238
13	18	1	—	3	—	4	0.2020	0	0	0.003
16*	18	3	—	3	—	6	0.1246	-0.3333	-0.042	0.003
149	18	1	—	3	—	4	0.2020	0	0	0.003
144	18	—	—	3	—	3	0.2523	0.3333	0.084	0.002
Total						39			1.194	10.432

* One child omitted as there is doubt of his taste reaction.

Only twelve of the thirty families whose parents are both recorded provide any information on linkage and for these

$$S(\lambda) = 1.194, \quad (6.1)$$

$$S(\kappa) = 10.432, \quad (6.2)$$

leading to the estimate

$$1 - 4x = 0.114 \pm 0.310. \quad (6.3)$$

Again the information exceeds the total obtained from all the data by the method of sib-pairs, the information obtainable from the first set of thirty families by the latter method being only about 12 % of that shown in (6.2). This quantity could be further augmented by the development of a scoring system for those families for which only one parent has been examined, but the position there is not as simple as for the $\text{Sex} \times MN$ linkage studied in § 5.

7. $ABO \times MN$

Making use of the principles of § 2 above, thirteen of the first thirty families can be utilized in a test of this linkage. The technique is very similar to that used in § 4 for testing $\text{Sex} \times ABO$. Table 10 shows full particulars of the scorings.

The most interesting family here is no. 13. The mating is $AMN \times BMN$ and the children two ABM , one ABN and one BMN . Two segregations are scorable, one for A and one for B ; for each the ABM children form class a and the ABN class c , the BMN child being of no use for the score. Since all scorable children are AB , both segregations are correctly classed as doubtful, though the presence of a non-scorable child of phenotype B ensures that the A parent was heterozygous. The heterozygosity probability for the B parent is based on four children and, from I, Table 25, is 0.5556. Scoring with $\frac{1}{2}pu_{11}$ the value of κ is $p^2\omega_1$; ω_1 being 3 in either case, the figures shown in Table 10 are obtained. Four of the segregations scored are of the new types discussed in § 2, but their treatment has been sufficiently illustrated in § 4.

Table 10. *Scoring of $ABO \times MN$*

Family	Type	a	b	c	d	Segre- gating	s	p	λ/p	λ	κ
119	19	1	—	1	1	A	3	1.0000	-0.2222	-0.222	2.198
88	17	—	2	—	—	A	2	1.0000	1.0000	1.000	1.000
132	16'	1	1	1	1	B	4	1.0000	-2.0000	-2.000	1.000
1	19	—	—	2	—	B	2	0.4941	0.2222	0.110	0.012
65	16'	—	1	—	1	B	2	1.0000	-1.0000	-1.000	1.000
147	18	—	—	3	—	A	3	0.4895	0.3333	0.163	0.009
139*	20'	2	—	—	2	B	4	1.0000	6.0000	6.000	6.000
14 ^b	18	—	1	2	1	B	4	1.0000	-0.8889	-0.889	0.941
13	17	2	—	1	—	A	3	1.0000	-1.0000	-1.000	3.000
13	17	2	—	1	—	B	3	0.5556	-1.0000	-0.556	0.926
16	16	3	—	4	—	B	7	0.1351	-3.0000	-0.405	0.383
130	20'	—	—	1	1	B	2	1.0000	-1.0000	-1.000	1.000
149	18	3	1	—	1	A	5	1.0000	-0.6667	-0.667	1.418
150	16	1	—	1	—	A	2	0.5556	-1.0000	-0.556	0.309
Total							43†			-1.022	19.196

* One child of this family omitted as possibly being a half-brother.

† Each family included *once* only.

Taking the appropriate totals, $S(\lambda) = -1.022$, (7.1)

$S(\kappa) = 19.196$, (7.2)

which give the estimate $1 - 4x = -0.053 \pm 0.228$. (7.3)

Table 4 only shows the information obtained by sib-pair scoring on the existence of an $A \times MN$ linkage, but the total given in (7.2) leaves little doubt of the superiority of the efficient scoring on the thirty families alone.

8. $ABO \times T$

The computations for the test of this linkage are more troublesome than for any of the other five. Matings of two test factors both of which show dominance require the consideration of two heterozygosity probabilities, p_0 and p_i ; some care is necessary in order that these may be calculated from the correct numbers of children and that the family may be placed in the correct certain-doubtful category. The detailed scoring of the fifteen families which contribute information is shown in Table 11.

Perhaps the most interesting family for discussion is no. 65. In this $ABT \times BT$ mating the children are two AT , one BT , and a B child of unspecified taste reaction. The A segregation, of type 18', is scorable for three children, with $a = 1$, $b = 2$. The BT parent is certainly heterozygous for B , and there is a probability of 0.2523 of both parents being heterozygous for T . For this segregation the value of $\frac{1}{18}u_{31}$ is $-\frac{1}{8}$ and the score is therefore -0.028 . Table 2 shows that the value of κ appropriate to this score is $\frac{1}{81}p_t^2\omega_1$, or 0.002. The B segregation is scored, for the two A children only, as type 14'. The score is easily found as 0.028; the variance for this type of doubtful segregation with $b = d = 0$ is $\frac{1}{81}p_t^2\omega_{13}$, or 0.001. In addition there must be added the information κ_0 , which for this type is $\pi_0\pi_t\gamma_6$. In this formula π_0 is based on four children and π_t on three, these being the numbers tested for the two characters. The values are therefore 0.5556 and 0.2523; the product of these with γ_6 gives κ_0 as 0.001.

Table 11. *Scoring of ABO \times T*

Family	Type	a	b	c	d	Segre- gating	s	p_0	p_t	λ/p_0p_t	λ	κ	κ_0
119	14	3	1	—	—	A	4	1.0000	0.1111	-0.6667	-0.074	0.008	0.001
120	16	—	1	1	—	—	2	1.0000	1.0000	1.0000	1.000	1.000	—
132	13'	1	2	1	—	B	4	1.0000	1.0000	0	0	6.133	0.013
1	14	—	—	7	—	A	7	0.4941	1.0000	2.3333	1.153	0.053	0.000
65	18'	1	—	2	—	A	3	1.0000	0.2523	-0.1111	-0.028	0.002	—
65	14'	—	—	2	—	B	2	1.0000	0.2523	0.1111	0.028	0.001	0.001
147	14	2	—	1	—	A	3	0.4895	1.0000	-0.1111	-0.054	0.006	0.001
148	14	2	—	—	—	A	2	0.5556	0.3103	0.1111	0.019	—	0.001
140	18	—	1	—	3	—	4	1.0000	1.0000	0	0	0.941	—
15	15	1	—	1	—	A	2	1.0000	0.3103	-0.0741	-0.023	0.001	0.000
12	14	1	—	—	1	A	2	1.0000	1.0000	0.3333	0.333	0.236	0.001
12	14	1	—	—	1	B	2	1.0000	0.3333	0.3333	0.333	0.236	0.001
13	14	3	—	1	—	A	4	1.0000	0.2020	0	0	0.002	0.001
13	14	4	—	—	—	B	4	0.5556	0.2020	0.6667	0.075	—	0.001
22	13	1	—	1	—	A	2	1.0000	0.3333	-1.0000	-0.333	0.099	0.009
16*	14	2	—	4	—	A	6	1.0000	0.1246	-0.1111	-0.014	0.002	0.000
16*	14	6	—	—	—	B	6	0.6346	0.1246	1.6667	0.133	—	0.000
149	15	2	—	2	—	A	4	1.0000	0.2020	-0.0494	-0.010	0.001	0.000
144	14	2	—	1	—	A	3	1.0000	0.2523	-0.1111	-0.028	0.002	0.001
Total							52†				2.510	8.723	0.031

* One child omitted as there is doubt of his taste reaction.

† Each family included *once* only.

As a further example, consider family 13, a mating of $AT \times BT$ having children three ABT and one BT . Both segregations are of type 14 and are scored on all children. In each the T segregation is classed as doubtful, the value of p_t being 0.2020; the presence of a B child makes the A segregation certain, and p_0 for the B segregation is 0.5556. The scores are immediately obtained as 0 and 0.075. The value of κ for type 14 with $b = d = 0$ is (I, Table 2) $\frac{1}{81}p_t^2\omega_{13}$ or 0.002, but the B segregation, being doubtful for both factors, does not contribute to this column. For each there must be added $\kappa_0 = \pi_0\pi_t\gamma_6$, π_0 for the A segregation being 0.2381 and the other values being the same as the corresponding values of p . In both cases γ_6 is 0.0137 and thus both entries for κ_0 are 0.001.

The total score is

$$S(\lambda) = 2.510, \quad (8.1)$$

with variance, divisor, and information

$$S(\kappa + \kappa_0) = 8.754. \quad (8.2)$$

Hence the linkage estimate is $1 - 4x = 0.287 \pm 0.338. \quad (8.3)$

The information is again probably larger than would be obtained by scoring both $A \times T$ and $B \times T$ linkages on the sib-pair system for all families, but it is unlikely that much increase would result from an efficient scoring of the incomplete families, since the information on this linkage obtainable from doubtful families is very small.

9. $MN \times T$

Only nine of the first thirty families contribute information on the possibility of linkage of this pair of factors, their scoring being very similar to that for $\text{Sex} \times T$. The only comment that need be made on the details of Table 12 is that for families 119 and 13 the values of p are based on four children, there being unscorable MN children in each family.

Table 12. *Scoring of $MN \times T$*

Family	Type	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>s</i>	<i>p</i>	λ/p	λ	κ
119	17	1	—	1	—	2	0.1111	-1.0000	-0.111	0.012
120	18	1	1	—	—	2	1.0000	-0.3333	-0.333	0.238
132	16	1	1	2	—	4	1.0000	0	0	6.000
1	17	—	—	—	2	2	1.0000	1.0000	1.000	1.000
65	18	2	—	1	—	3	0.2523	-0.1111	-0.028	0.002
148	18	2	—	—	—	2	0.3103	0.1111	0.034	0.001
13	19	2	—	1	—	3	0.2020	-0.2222	-0.045	0.006
16*	18	3	—	3	—	6	0.1246	-0.3333	-0.042	0.003
149	18	3	—	1	—	4	0.2020	0	0	0.003
Total						28			0.475	7.265

* One child omitted as there is doubt of his taste reaction.

From this table

$$S(\lambda) = 0.475, \quad (9.1)$$

$$S(\kappa) = 7.265, \quad (9.2)$$

and the estimate is

$$1 - 4x = 0.065 \pm 0.371. \quad (9.3)$$

Even though very few families are thus scorable, the information obtained is considerably greater than that shown in Table 4 for the whole data.

10. COMBINATION OF ESTIMATES, SUMMARY, AND CONCLUSIONS

Methods of efficient scoring for linkage tests on family data, which were catalogued in I for linkages of gene pairs, have in the present paper been extended to cover the mating types whose scoring is necessary in a test of a linkage with the ABO blood groups. The information obtained on six linkage tests applied to the thirty families, from data collected by Boyd, in which both parents are recorded is compared with that obtained from the whole data (202 families) by use of the Penrose sib-pair test. In every case the thirty

families alone, when efficiently scored, provide more information than the 500 pairs of sibs.

The technique of efficient scoring of families one of whose parents is unrecorded has not yet received any detailed analysis. Fisher has suggested an 'intermediate' score, involving only a small loss of efficiency, for certain mating types for which it is not certain which of two segregations has actually taken place. In the present paper two of the simplest cases occurring in tests of sex linkage have been examined, but no complete treatment for other matings can yet be given.

Penrose's method ignores any information provided by the parental records, and there is thus a very great loss of efficiency on the complete families. For families of which only one parent is recorded the loss of efficiency in Penrose's test is naturally less, though the one case examined in detail suggests that, at least for a sex linkage, the labour involved in devising efficient scoring systems may be repaid by a considerably increased precision. The families which consist only of sibs are probably satisfactorily scored by the sib-pair method,* though the correct weighting of families of different sizes needs closer attention than it has yet received.

As a first test of linkage on a body of data it is probably sufficient merely to score the complete families. If there is no suggestion of significance obtained thus, there is little need to proceed further. On the other hand, if there appears to be a possibility that the two factors under test may be linked, there are the alternatives of applying efficient scoring to families for which only one parent is recorded or of scoring all remaining pairs of sibs by Penrose's method. In the latter event it should be noted that no sibs should be scored from those families which fail to contribute to the efficient score on account of having no doubly heterozygous parent, as they contain no information. In § 3 it was shown how an estimate of $(1 - 4\xi)$ and a corresponding quantity of information may be derived from sib-pair score. The scores from the two portions of the data may then be combined, weighting the components according to their information content.

None of the six linkages tested give scores approaching significance for the thirty complete families, as has been shown in §§ 4-9. However, as an example of technique, the combination of the scores for Sex \times MN will be made here. In § 5 an efficient score has been obtained for this linkage test for all families of which the mother is recorded—whether or not the father is also given. In addition separate sib-pair scores have been assigned to eleven families in which only the father is recorded and to 137 families having no parental records. The 300 sib-pairs from both these classes may be combined in a single estimate, which is found to be

$$1 - 4x = -0.035,$$

the information being 6.645. From (5.25) and (5.26) the estimate of $(1 - 4x)$ from the efficiently scored families is 0.133 with information 41.844. The weighted mean of the two estimates, using the informations as weights, is

$$1 - 4x = 0.110,$$

* Further investigations (to be published later) throw doubt on the validity of this conclusion.

the total information now being 48.489, and therefore the standard error of the estimate ± 0.144 . Here, of course, there is little indication of linkage, but in data of this type, where many families are incompletely recorded, the information obtainable by scoring the less complete portions of the records by the Penrose method might form a very useful supplement to that from the families efficiently scored.

For comparison with Table 4, the results of the linkage tests in §§ 4-9 are summarized in Table 13. The figures shown are based only on those families which have been scored by u -statistics, without the addition of the information obtainable by scoring sib-pairs from the remainder of the records. The quantities of information given by certain and doubtful families have been totalled separately in order to illustrate how small a proportion of the total is provided by the doubtful category. Had the latter been rejected completely on account of lack of knowledge of gene frequencies the loss of information would have

Table 13. *Estimates of linkage and quantities of information*
(u -statistics applied to Boyd's data)

Linkage	$(1 - 4x)$	σ	$S(\kappa_c)$	$S(\kappa_d)$	$S(\kappa_c + \kappa_d)$
Sex \times ABO	-0.029	0.117	69.718	2.957	72.675
Sex \times MN	0.133	0.155	41.000	0.844	41.844
Sex \times T	0.114	0.310	10.417	0.015	10.432
ABO \times MN	-0.053	0.228	17.557	1.639	19.196
ABO \times T	0.287	0.338	8.561	0.193	8.754
MN \times T	0.065	0.371	7.238	0.027	7.265

been $8\frac{1}{2}\%$ on the ABO \times MN test and considerably less on the others, the average loss being about 4 %. This gives ample justification for the use of gene frequencies which may be somewhat in error for the population examined, since even moderately large changes in the estimates of these frequencies could have little effect on the final tests.

The only conclusion to be drawn from the data examined is that there is no indication of the existence of any genetic linkages between the ABO and MN blood groups and the factor associated with the capacity to taste phenyl-thiocarbamide, or between any of these factors and sex. None of the scores for the pairs of factors exceeds its standard error. In particular, the scoring of Sex \times T with greater efficiency disposes of the slight indication of linkage given by the Penrose test as applied by Boyd.

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THE DETECTION OF LINKAGE

III. INCOMPLETE PARENTAL TESTING

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I. INTRODUCTION

PREVIOUS papers of this series (Finney, 1940, 1941; references to these will in future be given as I and II) have shown the development and application of efficient tests for linkage in human family material extending over two generations. Unfortunately it frequently happens that it is impossible to obtain full descriptions of both parents for both the factors under test. In such cases it would be natural to have recourse to methods of test which are not fully efficient, either of the type given by Penrose (1938), the efficiency of which is unknown, or of the nature of the 'intermediate scores' introduced by Fisher (1935), whose efficiencies are known to be high.

The problem of devising efficient scores for families in which incomplete knowledge of parental phenotypes prevents the determination of the mating type involved was considered in II for a particularly simple case. In the present paper the methods introduced there are shown to be capable of extension to all situations in which the parental uncertainty relates to one only of the two genetic factors whose possibility of linkage is to be examined, provided that it is possible to estimate population gene frequencies relating to this factor. Such estimation need not be of very high accuracy, as its use is only to provide relative weights for the various components of a score.

The paper contains a generalization of the fundamental theorem of efficient scoring systems stated in I, this being necessary to the understanding of the inter-relationships of the different forms of the information function which may be assigned to a single family. This is followed by discussion of the situations arising from all the mating types considered in I when records of either factor are lacking for one or both parents. More complicated matings, such as those for the *ABO* blood groups treated in II, are not discussed here, but the method of analysis to be used for these will be apparent. The example of the new technique, which was used in II as an illustration of the gain in information to be obtained, is completed by the inclusion of all families in the records, and shows equally satisfactory increases for the newly included material.

It is unfortunately unavoidable that, as the genetic situation in the parental generation becomes more obscure through incompleteness of records, the technique of applying efficient tests of linkage becomes more complex. Nevertheless, once the fundamental principles become clear, it will be found that the formulae given here are not as intractable as might at first appear; the improvement in the precision of the results obtained by their use will frequently be large by comparison with the additional time spent in computing them.

2. THEORY OF ESTIMATION

The estimation theory necessary to an understanding of the theoretical basis of the method of scoring used in this series of papers was formally developed in I, § 2, where, however, only the simple case in which all families fall into a single group with no subgroups was considered. In that paper, and also in II, the practical advantages, in certain circumstances, of dividing families into subgroups, by means of ancillary information concerning them, were discussed, and it was found that the information on linkage from each group separately might then be supplemented by an amount resulting from the division into groups. It seems appropriate to show here the generalization of the earlier theory which justifies this procedure. The situation is closely allied to that of the Analysis of Variance.

Suppose a population which contains N different categories to be divided into a groups, group i containing N_i categories, so that

$$\sum_{i=1}^a N_i = N. \quad (2.1)$$

It is desired to test the null hypothesis $\theta = 0$, (2.2)

where P_{ij} , the probability of a single observation falling into category j of group i , is a function of the parameter θ . In the neighbourhood of $\theta = 0$,

$$P_{ij} = p_{ij}\{1 + \lambda_{ij}\theta + 0(\theta^2)\}, \quad (2.3)$$

where, since $\sum_{i=1}^a \sum_{j=1}^{N_i} P_{ij} = 1$ for all θ ,

$$\sum_i \sum_j p_{ij} = 1, \quad (2.4)$$

and

$$\sum_i \sum_j p_{ij} \lambda_{ij} = 0. \quad (2.5)$$

It will be convenient to write

$$\sum_j P_{ij} = P_i, \quad (2.6)$$

$$\sum_j p_{ij} = p_i, \quad (2.7)$$

$$\sum_j p_{ij} \lambda_{ij} = p_i \lambda_i, \quad (2.8)$$

so that

$$P_i = p_i\{1 + \lambda_i\theta + 0(\theta^2)\}. \quad (2.9)$$

The *relative* probabilities of the N_i categories of group i are then seen to be

$$\frac{P_{ij}}{p_i} = \frac{p_{ij}}{p_i} \{1 + (\lambda_{ij} - \lambda_i)\theta + 0(\theta^2)\}. \quad (2.10)$$

If a sample of n independent observations is taken from the population, such that n_i fall into group i , it is clear that, just as in I, the efficient score for the detecting of departures of θ from zero is $S(\lambda)$, S denoting summation over the sample. Also the variance of, and information with respect to θ contained by, this score is $n\kappa_e$, where

$$\kappa_e = \sum_i \sum_j p_{ij} \lambda_{ij}^2. \quad (2.11)$$

An alternative approach is first to consider the information on θ given by the observations in each of the groups separately, as indicated by the variation within that group only.

If S_i denotes summation over the n_i observations in group i , it is seen from (2.10) that the score to be used for this group is $S_i(\lambda_{ij} - \lambda_i)$, and the information $n_i \kappa_i$, where

$$\kappa_i = \frac{1}{p_i} \sum_j p_{ij} (\lambda_{ij} - \lambda_i)^2 = \frac{1}{p_i} \sum_j p_{ij} \lambda_{ij}^2 - \lambda_i^2. \quad (2.12)$$

Thus separate tests may be made for each group, or they may be combined into a single test.

But (2.9) shows that, unless all the λ_i are zero, information on θ is also obtainable from the distribution of the n observations into the a categories. The score for this is $S(\lambda_i)$ and the information $n\kappa_0$, where

$$\kappa_0 = \sum_i p_i \lambda_i^2. \quad (2.13)$$

The total score for all observations is thus

$$\sum_i S_i(\lambda_{ij} - \lambda_i) + S(\lambda_i) = S(\lambda_{ij}), \quad (2.14)$$

which is identical with the score previously shown for the sample taken as a whole. The information contained by this score is

$$\sum_i n_i \kappa_i + n_0 \kappa_0 = \sum_i \frac{n_i}{p_i} \sum_j p_{ij} \lambda_{ij}^2 + \sum_i (np_i - n_i) \lambda_i^2. \quad (2.15)$$

At first sight this quantity looks very different from $n\kappa_c$. It may be noted, however, that if the null hypothesis be true

$$E(n_i) = np_i, \quad (2.16)$$

and therefore

$$E\left\{\sum_i n_i \kappa_i + n_0 \kappa_0\right\} = n \sum_i \sum_j p_{ij} \lambda_{ij}^2 = n\kappa_c. \quad (2.17)$$

The use of the second system of obtaining the total information differs from the first only in that it makes use of ancillary information or, alternatively, that it leads to the observed quantity of information on θ instead of the expected (Fisher, 1938, § 57.1). Whatever the form of classification used, the estimate T , of θ , is given by

$$TS(\kappa) = S(\lambda). \quad (2.18)$$

It is this more general theorem that is used in I when it is advised that 'certain' and 'doubtful' families be scored separately, in order that the heterozygosity probabilities of the parents may enter the expression for information in simpler manner and that the same formulae may apply when lack of information on gene frequencies compels the rejection of doubtful families. For example, in the discussion of the mating $Ttww \times TtWw$ given in § 8 of I the whole population of families of size s is divided into four classes, namely those families having both t and w genotypes present among the children, those lacking t but not w , those lacking w but not t , and those having TW children only. The value of κ is then found separately within each group, κ_c for the first group of certain heterozygosity, two forms of κ_d for the doubtful families of the next two groups, and zero for the families with only TW children. The total probabilities associated with these four groups are not independent of $(1 - 4\xi)$ and further information is obtained from this classification, the amount per family being the value of κ_0 shown in (8.20) of I. In some similar instances the value of κ_0 is zero, since the probabilities of the groups are independent of $(1 - 4\xi)$.

The classification of families into certain and doubtful categories adopted in I and II may now be seen to be a convenient way of obtaining the information rather than to indicate essential differences between the categories. This explains the apparent paradox of the statement in II that a family may be classed as doubtful on the evidence of its scorable children and yet other non-scorable children may establish as certain the heterozygosity of a parent or parents. Though it will frequently happen that the parental genotypes of a so-called doubtful segregation are uncertain, it must not be assumed from the nomenclature adopted that this is necessarily the case. For want of a more apt terminology, the use of 'certain' and 'doubtful' will continue in the present paper, though, in the literal sense, almost all the families discussed suffer in some degree from parental uncertainty.

3. PROBABILITIES OF PARENTAL GENOTYPES

When the genotypes of one or both parents with respect to any factor are unknown it is necessary to estimate from all the relevant data the probabilities of the possible alternatives. In general this must be done from knowledge of the phenotypes of the children observed. Though other relatives can also give information on this matter it seldom happens that there are observations available on them, and probably little will be lost by using only the children unless descriptions of other relatives are exceptionally full. It is not proposed to treat such exceptional cases here, but for the purposes of the present paper it is necessary to develop a number of formulae representing these probabilities as based on the children only. The simplest cases have been considered in § 4 of I; others are as follows, the symbol ? denoting an individual whose phenotype is unknown.

(i) 'Dominant' abnormalities.

? \times D . The dominant gene is so rare that it may be assumed that the unknown parent is d and the other heterozygous Dd .

$d \times ?$. If there are no D children both parents may be assumed to be d ; if abnormal children are present, the unknown must be taken as Dd .

? \times ?. Again if there are no D children both parents are taken to be d ; if D children are present, the mating is assumed $dd \times Dd$. In the latter case the probability that a particular parent is the abnormal one is $\frac{1}{2}$.

These three cases may all be obtained from group (iii) below by taking in the formulae there the frequency, T , of the dominant gene to be zero.

(ii) Recessive abnormalities.

The three cases in this group are exactly similar to those of group (iii).

(iii) Test factors showing dominance.

Take T , t as the gene frequencies of the dominant and recessive genes respectively, and suppose that in any family there are x dominant and y recessive children. Then the probability that a T individual is heterozygous is

$$v = 2t/(T + 2t), \quad (3.1)$$

this being the ν in which the heterozygosity probabilities of I were expressed. With this notation these become

$$p = \frac{t}{t + 2^{x-1}T} \quad (3.2)$$

for the probability that the T parent of a $T \times t$ mating is heterozygous when $y = 0$, and

$$p = \frac{3^x t^2}{3^x t^2 + 4^{x-1} T(T + 4t)} \quad (3.3)$$

for the probability that both parents of a $T \times T$ mating are heterozygous when $y = 0$.

? $\times T$. If $x \neq 0$, $y \neq 0$, the possible matings are $tt \times Tt$ and $Tt \times Tt$. Without knowledge of the children, the probabilities of these in a random mating population are respectively t^2 and $2Tt$. The probabilities of obtaining a family of the given constitution from these matings are proportional to 3^x and 2^{x+y} respectively. It follows that the required probabilities are

$$P(tt \times Tt) = \frac{2^{x+y-1}t}{3^x T + 2^{x+y-1}t}, \quad (3.4)$$

$$P(Tt \times Tt) = \frac{3^x T}{3^x T + 2^{x+y-1}t}. \quad (3.5)$$

If $x = 0$, $y \neq 0$, the same formulae apply. If $x \neq 0$, $y = 0$, there is no certainty that the T parent is heterozygous and there are three additional possible matings, none of which is of any value for linkage purposes. The probabilities are then

$$P(tt \times Tt) = \frac{2^{x-1}t^3}{4^{x-1}T(1 + 2Tt) + 3^x Tt^2 + 2^{x-1}t^3}, \quad (3.6)$$

$$P(Tt \times Tt) = \frac{3^x Tt^2}{4^{x-1}T(1 + 2Tt) + 3^x Tt^2 + 2^{x-1}t^3}. \quad (3.7)$$

$t \times ?$. If $x \neq 0$, $y \neq 0$, it follows that the missing parent must be Tt . If $x = 0$, $y \neq 0$, this parent may be either Tt or tt , and

$$P(Tt) = \frac{T}{T + 2^{y-1}t}, \quad (3.8)$$

similarly, if $x = 0$, $y \neq 0$, the missing parent is either Tt or TT , and

$$P(Tt) = \frac{t}{t + 2^{x-1}T}. \quad (3.9)$$

? $\times ?$. If $x \neq 0$, $y \neq 0$, there are only two possible matings, with probabilities

$$P(tt \times Tt) = \frac{2^{x+y}t}{3^x T + 2^{x+y}t}, \quad (3.10)$$

$$P(Tt \times Tt) = \frac{3^x T}{3^x T + 2^{x+y}t}. \quad (3.11)$$

If, however $x = 0$, the possibility that both parents are homozygous recessives must be considered, and it may be shown that

$$P(tt \times Tt) = \frac{2^y Tt}{T^2 + 2^y Tt + 4^{y-1}t^2}, \quad (3.12)$$

$$P(Tt \times Tt) = \frac{T^2}{T^2 + 2^y Tt + 4^{y-1}t^2}. \quad (3.13)$$

Again, if $y = 0$, there are a number of possibilities for the parental genotypes, those which are of interest for linkage studies having probabilities

$$P(tt \times Tt) = \frac{2^x t^3}{4^{x-1}(T^3 + 4T^2t + 2Tt^2) + 3^x Tt^2 + 2^x t^3}, \quad (3.14)$$

$$P(Tt \times Tt) = \frac{3^x Tt^2}{4^{x-1}(T^3 + 4T^2t + 2Tt^2) + 3^x Tt^2 + 2^x t^3}. \quad (3.15)$$

It must be remembered that in estimating the probability that a mating $? \times ?$ is $tt \times Tt$, the probability that a particular parent is the heterozygote is one-half the value given by (3.10), (3.12) or (3.14).

(iv) *Test factors showing no dominance.*

Take m, n as the gene frequencies of the two genes, and suppose that in any family there are xM , yMN , and zN children. When both parents have been observed, the genotypes are completely known, but if one or both parents are missing a number of cases for estimation of probabilities arise.

$? \times M$. Of necessity, $z = 0$. If $x \neq 0, y \neq 0$, the missing parent must be MN . If $y = 0$,

$$P(MN) = \frac{n}{2^{x-1}m + n}. \quad (3.16)$$

If $x = 0$,

$$P(MN) = \frac{m}{m + 2^{y-1}n}. \quad (3.17)$$

$? \times N$. The formulae here are simply obtained by interchanging m and n and also x and z in the previous paragraph.

$MN \times ?$. If $x \neq 0, z \neq 0$, the missing parent is MN . If $x \neq 0, z = 0$, it may be M or MN , and

$$P(M) = \frac{2^{x-1}m}{2^{x-1}m + n}, \quad (3.18)$$

$$P(MN) = \frac{n}{2^{x-1}m + n}. \quad (3.19)$$

Similarly, if $x = 0, z \neq 0$,

$$P(MN) = \frac{m}{m + 2^{z-1}n}, \quad (3.20)$$

$$P(N) = \frac{2^{z-1}n}{m + 2^{z-1}n}. \quad (3.21)$$

If $x = z = 0$, and thus only MN children are present,

$$P(M) = m^2, \quad (3.22)$$

$$P(MN) = 2mn, \quad (3.23)$$

$$P(N) = n^2. \quad (3.24)$$

$? \times ?$. Here there are numerous possible situations. If $x \neq 0, z \neq 0$, the mating must be $MN \times MN$. If $x \neq 0, y \neq 0, z = 0$, the two possible matings have probabilities

$$P(M \times MN) = \frac{2^x m}{2^x m + n}, \quad (3.25)$$

$$P(MN \times MN) = \frac{n}{2^x m + n}, \quad (3.26)$$

and symmetric results hold for the case of $x = 0, y \neq 0, z \neq 0$. Again, if $x \neq 0, y = z = 0$, $M \times M$ joins the list of possible matings, and those of interest for linkage have probabilities

$$P(M \times MN) = \frac{2^x mn}{4^{x-1} m^2 + 2^x mn + n^2}, \quad (3-27)$$

$$P(MN \times MN) = \frac{n^2}{4^{x-1} m^2 + 2^x mn + n^2}, \quad (3-28)$$

the results for $x = y = 0, z \neq 0$ being similar. Finally, if $x = z = 0, y \neq 0$,

$$P(M \times MN) = \frac{m^2}{m^2 + (1 + 2^{y-1}) mn + n^2}, \quad (3-29)$$

$$P(MN \times MN) = \frac{mn}{m^2 + (1 + 2^{y-1}) mn + n^2}, \quad (3-30)$$

$$P(N \times MN) = \frac{n^2}{m^2 + (1 + 2^{y-1}) mn + n^2}. \quad (3-31)$$

Once again it should be noted that, for $? \times ?$, the probability that the mating is $M \times MN$ or $N \times MN$ must be halved to obtain the probability that a particular parent is the heterozygote.

The derivation of the formulae listed above presents no great difficulty. Their expressions have a formidable appearance which is largely removed when numerical values are substituted for the gene frequencies. In practice it will be sufficient to use quite rough approximations to these frequencies, as, in any test of linkage, slight inaccuracies in the weighting of scores will produce only a very small loss of efficiency. It is not proposed to give at present any extensive tables of these probabilities, but these should be computed for the factors under test, and for small numbers of children, before scoring a collection of data. An example of this will be given later. Nor is any attempt made here to develop corresponding probabilities for factors dependent on multiple allelomorphs; for these there would obviously be many more cases to consider and the resulting formulae would be more complex.

4. LINKAGE OF 'DOMINANT' ABNORMALITIES

The enumeration of mating types will be based on that given in Table 1 of I. The addition of a or b to the type numbers of that table will indicate lack of knowledge with regard to the first or the second factor respectively; thus type $1a$ is $?d \times ?D$, where the $?, ?$ represent genotypes of the T, t series. In all cases it will be found desirable to make use of the ancillary information provided by the numbers of children of the possible phenotypes with reference to the unknown parental factor. This procedure is analogous to the use of (s_1, s_2) scoring systems for recessive abnormalities, and the scores and information functions obtained are generalizations of those which would result from the use for all mating types of this method of classification of families.

These functions, in their general algebraic form, are mostly rather cumbersome, though once their mode of construction is understood it is seen that they are quite manageable in

practice. The method of derivation of the efficient scores exemplified in I shows that, when it is not known which of two or more possibilities was actually the parental mating, the appropriate score is simply constructed by multiplying the score which would be attached to each of these possibilities by the probability of that mating being the correct one and summing for all the alternatives. The form of the information function to be attributed to this score is less obvious. Any one component of the score may be an *inefficient* score for any of the matings alternative to the one for which it is *efficient*, and the total information will thus generally be greater than the sum of the quantities corresponding to the separate components, being augmented by terms resulting from correlations of scores from alternative matings.

It is not proposed to tabulate the results in the same detail as was given in I, but it is necessary to comment on each mating type separately in order to make clear the method of scoring.

Types 1a and 2a: $d \times ?D$. These two types are identical. Information on linkage is obtainable from matings $td \times TD$ and $Td \times TD$ and the probabilities that a given family having x T and y t children is actually one or other of these may be assessed as in § 3. If these probabilities are p_1 and p_2 respectively, the score to be attached to the family is

$$\lambda = \frac{1}{2} p_1 u_{11} + \frac{1}{18} p_2 u_{31} = p_1 \lambda_1 + p_2 \lambda_2, \quad (4.1)$$

where λ_1, λ_2 are the scores which would be used if it were known that the mating was type 1 or type 2 respectively. If a, b, c, d are defined as in Table 1 of I, then

$$a + c = x, \quad (4.2)$$

$$b + d = y, \quad (4.3)$$

and

$$x + y = s. \quad (4.4)$$

It should be noted that here, as always in similar circumstances, the x and y used in the score and its associated information content are not necessarily identical with the values from which p_1 and p_2 are determined. For the determination of these probabilities all children described for their test-factor should be employed, whether or not their phenotype with respect to the abnormality is known, but children for which this phenotype is unknown cannot of course be scored for linkage purposes.

The frequencies of families of different constitutions, but having x, y fixed, for either type 1 or type 2 in the absence of linkage will be proportional to the coefficients in the expansion of

$$\Phi = (\alpha + \gamma)^x (\beta + \delta)^y. \quad (4.5)$$

Now the operator which leads to the mean and variance of the score is clearly

$$D = \frac{1}{2} p_1 D_{11} + \frac{1}{18} p_2 D_{31}, \quad (4.6)$$

where D_{11} and D_{31} are the simple forms of operator defined in (5.5) and (6.3) of I. Applying these in the usual way it appears that

$$D_{11}^2 \Phi = 2^{s+1} \{(x+y)^2 - (x+y)\}, \quad (4.7)$$

$$D_{11} D_{31} \Phi = 2^{s+1} \{(x+3y)^2 - (x+9y)\}, \quad (4.8)$$

and

$$D_{31}^2 \Phi = 2^{s+1} \{(x+9y)^2 - (x+81y)\}, \quad (4.9)$$

after putting $\alpha = \beta = \gamma = \delta = 1$. The frequency with which functions similar to these appear suggests the introduction of the notation

$$v_a(x, y) = (x + ay)^2 - (x + a^2y), \quad (4.10)$$

which will be written simply as v_a in cases where no confusion is possible. Combining these results and remembering that the variance of the score in the other possible mating types, which could of themselves throw no light on linkage, is zero, the general theorem shows the variance, information, and score divisor to be

$$\begin{aligned} \kappa &= 2^{-s} D^2 \Phi, \quad \text{with } \alpha = \beta = \gamma = \delta = 1, \\ &= \frac{1}{2} [p_1^2 v_1 + \frac{2}{9} p_1 p_2 v_3 + \frac{1}{81} p_2^2 v_9]. \end{aligned} \quad (4.11)$$

It may be seen that this expression reduces to the value for type 1 when $p_1 = 1$, $p_2 = 0$, and to the value for type 2 when $p_1 = 0$, $p_2 = 1$, or rather to the values of κ for these matings which are obtained when the ancillary information given by x, y is used. The general formulae of (4.1) and (4.11) apply equally well whether one or both parents are not described for the test factor, provided that the appropriate expressions for the probabilities are selected from § 3.

Type 1b: $t? \times T?$. The only case to be considered from the linkage point of view is that in which the actual mating is $td \times TD$. Knowledge of the children enables the probability, p_d , of this mating to be estimated and the efficient score is

$$\lambda = \frac{1}{2} p_t p_d u_{11}, \quad (4.12)$$

with

$$\kappa = p_t^2 p_d^2 \omega_1, \quad (4.13)$$

where p_t is the probability of heterozygosity of the T parent derived from (3.2).

Type 2b: $T? \times T?$. If the probability that the mating is actually $Td \times TD$ is p_d , allowance being made for either parent to be the abnormal one, and the probability that both parents are heterozygous Tt is p_t , the score is found to be

$$\lambda = \frac{1}{18} p_t p_d u_{31}, \quad (4.14)$$

and

$$\kappa = p_t^2 p_d^2 \omega_2. \quad (4.15)$$

Types 3a and 4a: $?d \times ?D$. The possible matings which are of interest from the point of view of linkage are $Md \times MND$, $MNd \times MND$, and $Nd \times MND$; the probabilities, p_1, p_2, p_3 respectively, that the mating observed is one of these three may be assessed as in § 3 from the knowledge that there are x M , y MN , and z N children. If the scores appropriate to these three matings are $\lambda_1, \lambda_2, \lambda_3$, the first and last being of type 2 and the second of type 4, the score for type 3a is

$$\lambda = p_1 \lambda_1 + p_2 \lambda_2 + p_3 \lambda_3. \quad (4.16)$$

The usual process shows the information to be

$$\begin{aligned} \kappa &= \frac{1}{2} [p_1^2 v_1(x, y) + p_2^2 v_1(x, z) + p_3^2 v_1(y, z) \\ &\quad + 2p_1 p_2 v_1(x, 0) + 2p_1 p_3 v_1(y, 0) + 2p_2 p_3 v_1(z, 0)]. \end{aligned} \quad (4.17)$$

This expression must always simplify to some extent, since if there are M children $p_3 = 0$, if there are N children $p_1 = 0$, and if there are only MN children $x = y = 0$.

Type 3b: $M? \times MN?$. The only probability to be estimated is that the mating is actually type 3; if this is p ,

$$\lambda = \frac{1}{2}pu_{11}, \quad (4.18)$$

and

$$\kappa = p^2\omega_1. \quad (4.19)$$

Type 4b: $MN? \times MN?$. If p is the probability that the mating is really of type 4, allowance being made for either parent to be the Dd heterozygote, MN children are rejected from the scoring and

$$\lambda = \frac{1}{2}pu_{11}, \quad (4.20)$$

and

$$\kappa = p^2\omega_1. \quad (4.21)$$

5. LINKAGE OF RECESSIVE ABNORMALITIES

When the parental uncertainty is in respect of the test factor, development of the appropriate expressions for information on the lines of the previous section leads to algebraic formulae which it is not proposed to consider fully at present, as they are probably too complicated for any frequent use to be made of them. Cases of unknown ascertainment procedure, for which (s_1, s_2) scoring systems would normally be used, would in any case fall outside the scope of this paper, since their analysis requires the consideration of families with fixed numbers of children in the phenotypic categories of both factors.

There is, however, no difficulty in determining the efficient score to be used for this series of matings, since it is always of the form $S(p\lambda)$, where λ is the score for any mating which is possible when the observations on the children are taken into account, p is the probability, assessed as in § 3, that this mating is the true one, and S denotes summation over all such possibilities. The variance of these scores for families of given size may then be estimated empirically from the actual values obtained in any given body of data. The variance of the total score of these families must clearly be an empirical estimate of the information contained in them, and scores and informations from families of differing size may be added in the usual way. This method should be compared with the suggestion made by Fisher (1935, § VI), for the use of an 'intermediate' type of score when there is doubt as to whether u_{31} or u_{33} is correct. Though the efficiency of this can be made to be almost 90 %, there seems little reason to prefer it to the efficient score, as there cannot be any great reduction in computational time resulting from the use of it. Indeed the efficient score can probably be computed more rapidly in practice, and has also the advantage of greater generality, in that it is still applicable when more than two parental combinations are possible.

The method of obtaining an empirical variance is, however, exactly that given by Fisher, and his suggestion for treating a small number of large families may also be adopted. This use of an empirical variance does, in fact, give an efficient method of test for cases where the lack of parental descriptions extends to both factors, whatever the mating types involved, and may thus be used for families other than those considered in the present paper.

The b series of mating types, for which the parental uncertainty is for the abnormality, needs no further comment here, as the results are exactly as for corresponding cases of linkages of two test factors, attention being confined to cases of unknown ascertainment since the numbers of dominant and recessive children are to be used as ancillary information.

6. LINKAGE OF TWO TEST FACTORS, BOTH SHOWING DOMINANCE

Types 13a and 14a: ?w × ?W. If both parents were described for the first test factor, information on linkage would be given by types 13 and 14; λ_1 and λ_2 being the scores appropriate to these two types, the score for type 13a is therefore

$$\lambda = p_1 \lambda_1 + p_2 \lambda_2 = \frac{1}{2} p p_1 u_{11} + \frac{1}{18} p p_2 u_{31}, \quad (6.1)$$

where p_1 and p_2 are the probabilities of the parents being tt , Tt or Tt , Tt respectively, and p is the probability that the W parent is heterozygous Ww . For families certain for W the generating function is

$$\Phi = (\alpha + \gamma)^x (\beta + \delta)^y - \alpha^x \beta^y, \quad (6.2)$$

and thus the total frequency $\Sigma f = 2^s - 1$. (6.3)

The operator to be used is $D = \frac{1}{2} p_1 D_{11} + \frac{1}{18} p_2 D_{31}$. (6.4)

After differentiation and the putting of $\alpha = \beta = \gamma = \delta = 1$,

$$D_{11} \Phi = -v_{-1}, \quad D_{31} \Phi = -v_{-3},$$

and therefore $\bar{\lambda} = -\frac{1}{2(2^s - 1)} (p_1 v_{-1} + \frac{1}{9} p_2 v_{-3})$. (6.5)

A second differentiation, again followed by putting $\alpha = \beta = \gamma = \delta = 1$, shows that

$$D_{11}^2 \Phi = 2^{s+1} v_{-1} - v_{-1}^2, \quad D_{31} D_{11} \Phi = 2^{s+1} v_{-3} - v_{-1} v_{-3}, \quad D_{31}^2 \Phi = 2^{s+1} v_{-3} - v_{-3}^2,$$

whence it is found that

$$\kappa = \frac{1}{2(2^s - 1)} [2^s (p_1^2 v_{-1} + \frac{2}{9} p_1 p_2 v_{-3} + \frac{1}{81} p_2^2 v_{-3}) - \frac{1}{2} (p_1 v_{-1} + \frac{1}{9} p_2 v_{-3})^2] - \bar{\lambda}^2. \quad (6.6)$$

The analogy between the value of $\bar{\lambda}$ given by (6.5) and ϵ_8 and ϵ_{10} should be noted, as also that between the value of κ in (6.6) and ω_8 , ω_{10} ; in the first case the more general formula of the present paper is a suitably weighted sum of the corresponding expressions introduced in I, and in the second there is also added an intermediate correlation term. Families doubtful for W (i.e. having no w children) of themselves contribute no information, but once again information accrues from the classification of certain and doubtful families. The score for this purpose has simply the effect of making unnecessary the use of score corrections, $\bar{\lambda}$, and the information is

$$\kappa_0 = \frac{\pi}{4(2^s - 1)} (p_1 v_{-1} + \frac{1}{9} p_2 v_{-3})^2, \quad (6.7)$$

again closely analogous to the corresponding expressions for types 13 and 14.

Type 15a: ?W × ?W. Here the probabilities, p_1 and p_2 , that the parents are either one or both Tt must be estimated; the first leads to a complete mating of type 14 (with the two factors interchanged in the standard definitions), the second to type 15. Having assigned the children to the four phenotypic classes, the score is

$$\lambda = \frac{1}{18} p p_1 u_{31} + \frac{1}{81} p p_2 u_{33}, \quad (6.8)$$

p being the heterozygosity probability for W . By processes very similar to those used for type 13a, it may be shown that, for families certain for W (i.e. having at least one w child),

$$\bar{\lambda} = -\frac{3^{s-2}}{2(4^s - 3^s)}(p_1 v_{-1} + \frac{2}{3} p_2 v_{-3}) \quad (6.9)$$

and
$$\kappa = \frac{1}{18(4^s - 3^s)}[4^s(p_1^2 v_1 + \frac{4}{9} p_1 p_2 v_3 + \frac{4}{81} p_2^2 v_9) - \frac{1}{2} 3^{s-2}(p_1 v_{-1} + \frac{2}{3} p_2 v_{-3})^2] - \bar{\lambda}^2. \quad (6.10)$$

Once more the use of families doubtful for W cancels the score correction, $\bar{\lambda}$, and introduces an amount of information per family (certain and doubtful)

$$\kappa_0 = \frac{\pi 3^{s-4}}{4(4^s - 3^s)}(p_1 v_{-1} + \frac{2}{3} p_2 v_{-3})^2. \quad (6.11)$$

The analogies between (6.9) and e_9 , e_{11} , (6.10) and ω_9 , ω_{11} , (6.11) and $\pi\gamma_9$, $\pi\gamma_{11}$ may be noted.

Nothing need be said of *Type 13b* except that it is symmetric with type 13a, nor of *Types 14b and 15b* (which are identical) except that they are symmetric with type 15a. The reader who has followed the derivation of expected quantities of information, κ_e , taking together certain and doubtful families, as given in I, will readily see how the results may be extended to the matings considered in this section; it seems unnecessary to quote the formulae as it is unlikely that they will be required in practice.

7. LINKAGE OF TWO TEST FACTORS, ONE SHOWING DOMINANCE

Types 16a and 17a: ?w × ?W. If p_1 , p_2 , and p_3 are the probabilities that the W parent is MN and the other M , MN , or N respectively, the score is

$$\lambda = p_1 \lambda_1 + p_2 \lambda_2 + p_3 \lambda_3, \quad (7.1)$$

λ_1 , λ_2 , λ_3 being the scores appropriate to these three complete matings, the first and last of type 16 and the second of type 17. Using the ancillary information that there are x M , y MN , and z N children, it may be shown that, for families certain for W ,

$$\bar{\lambda} = -\frac{1}{2(2^s - 1)}\{p_1 v_{-1}(x, y) + p_2 v_{-1}(x, z) + p_3 v_{-1}(y, z)\} = p_1 \bar{\lambda}_1 + p_2 \bar{\lambda}_2 + p_3 \bar{\lambda}_3, \quad (7.2)$$

and
$$\kappa = \frac{1}{2(2^s - 1)}[2^s\{p_1^2 v_1(x, y) + p_2^2 v_1(x, z) + p_3^2 v_1(y, z) + 2p_1 p_2 v_1(x, 0) + 2p_1 p_3 v_1(y, 0) + 2p_2 p_3 v_1(z, 0)\} - \frac{1}{2}\{p_1 v_{-1}(x, y) + p_2 v_{-1}(x, z) + p_3 v_{-1}(y, z)\}^2] - \bar{\lambda}^2. \quad (7.3)$$

There is no information from those families doubtful for W , but from the certain-doubtful classification is obtained

$$\kappa_0 = \frac{\pi}{4(2^s - 1)}\{p_1 v_{-1}(x, y) + p_2 v_{-1}(x, z) + p_3 v_{-1}(y, z)\}^2. \quad (7.4)$$

Types 18a and 19a: ?W × ?W. With p_1 , p_2 , and p_3 defined as above, except that allowance must now be made for either parent to be doubly heterozygous,

$$\lambda = p_1 \lambda_1 + p_2 \lambda_2 + p_3 \lambda_3, \quad (7.5)$$

where λ_1 and λ_3 are scores of the form $\frac{1}{18}u_{31}$ for type 18 matings and λ_2 of the form $\frac{1}{6}u_{31}$ for a type 19. For the families certain for W

$$\bar{\lambda} = p_1\bar{\lambda}_1 + p_2\bar{\lambda}_2 + p_3\bar{\lambda}_3 \quad (7.6)$$

$$\text{and } \kappa = \frac{1}{18(4^s - 3^s)} [4^s\{p_1^2 v_1(x, y) + 4p_2^2 v_1(x, z) + p_3^2 v_1(y, z) + 4p_1 p_2 v_1(x, 0) + 2p_1 p_3 v_1(y, 0) + 4p_2 p_3 v_1(z, 0)\} - \frac{1}{2}3^{s-2}\{p_1 v_{-1}(x, y) + 2p_2 v_{-1}(x, z) + p_3 v_{-1}(y, z)\}^2] - \bar{\lambda}^2. \quad (7.7)$$

Once again the information from the families doubtful for W is zero, but

$$\kappa_0 = \frac{\pi 3^{s-4}}{4(4^s - 3^s)} \{p_1 v_{-1}(x, y) + 2p_2 v_{-1}(x, z) + p_3 v_{-1}(y, z)\}^2. \quad (7.8)$$

Types 16b and 18b: $M? \times MN?$. The analysis for this is exactly as for type 1a, MN replacing D and M replacing d .

Types 17b and 19b: $MN? \times MN?$. Again the analysis is very similar to that for type 1a, but a slight difference arises from the fact that the score for type 19 is $\frac{1}{6}u_{31}$, whereas that for type 2 is $\frac{1}{18}u_{31}$. If p_1 is the probability that the complete mating is $MNw \times MNWw$, and p_2 the probability that it is $MNWw \times MNWw$ the possibility of either parent being the Ww being allowed in the former case, and the probabilities being computed from all children, the score is

$$\lambda = \frac{1}{2}p_1 u_{11} + \frac{1}{6}p_2 u_{31} = p_1 \lambda_1 + p_2 \lambda_2, \quad (7.9)$$

λ_1 and λ_2 being scores for type 17 and type 19 matings respectively, for both of which, of course, MN children are rejected. Then

$$\kappa = \frac{1}{2}(p_1^2 v_1 + \frac{4}{9}p_1 p_2 v_3 + \frac{4}{81}p_2^2 v_9). \quad (7.10)$$

8. LINKAGE OF TWO TEST FACTORS, NEITHER SHOWING DOMINANCE

Type 20a and 21a: $?P \times ?PQ$. For this type the scoring is exactly as for type 3a, with PQ replacing D and P replacing d .

Type 22a: $?PQ \times ?PQ$. This bears the same relation to type 3a as does type 17b to type 1a. If p_1, p_2 , and p_3 are the probabilities, based on all children, that the complete mating is $MPQ \times MNPQ$, $MNPQ \times MNPQ$, or $NPQ \times MNPQ$ respectively, the first and third being symmetric with type 21 and the second being type 22, the score is

$$\lambda = p_1 \lambda_1 + p_2 \lambda_2 + p_3 \lambda_3. \quad (8.1)$$

In this expression λ_1 and λ_2 are of the form $\frac{1}{2}u_{11}$, and λ_3 of the form u_{11} . The information content of the score is

$$\kappa = \frac{1}{2}\{p_1^2 v_1(x, y) + 4p_2^2 v_1(x, z) + p_3^2 v_1(y, z) + 4p_1 p_2 v_1(x, 0) + 2p_1 p_3 v_1(y, 0) + 4p_2 p_3 v_1(z, 0)\}. \quad (8.2)$$

This completes the discussion of the matings of this section, since *Type 20b* is symmetric with type 20a, and *Types 21b and 22b* are identical and symmetric with type 22a.

9. THE TECHNIQUE OF SCORING AND A COMPARISON OF QUANTITIES OF INFORMATION

When the suggestion of scoring for linkage families whose complete mating types were not known was introduced in § 5 of II, an example was given of the arithmetical procedure involved. This was chosen as the particularly simple case of a $\text{Sex} \times MN$ linkage test, using only the families for which the father's MN type was unknown and the mother's known. For the more complex situations discussed in the present paper, the arithmetic becomes a little more laborious, though it is less troublesome than the formulae shown earlier might suggest.

The data collected by Boyd & Boyd (1941) will again be used to illustrate the processes. Unless an empirical estimation of variance is adopted the application must still be limited to families for which one parental factor is entirely known; it is convenient to take once more the $\text{Sex} \times MN$ test and to discuss the scoring for all the families in Boyd's records. Once again it will be assumed that the frequencies of the M and N genes are equal, or, in the notation of § 3, that $m = n = \frac{1}{2}$. More accurate values for these frequencies might be obtained from the records themselves, using the methods indicated by Fisher (1940), which are themselves related to the theory of scoring on which this series of papers is based. As has been said before, the attainment of greater accuracy here can scarcely alter the linkage scoring very substantially, representing as it does only comparatively small changes in weighting coefficients.

In scoring any large body of data, it will be found well worth while to tabulate, for families of moderate size, the relevant probability functions developed in § 3. Thus, (3.16) is seen to become

$$P(MN) = \frac{1}{2^{x-1} + 1}, \quad (9.1)$$

and this, together with (3.17)–(3.21), (3.25) and (3.26), may be obtained from the first column of Table 25 of I, which is a tabulation of

$$p = \frac{1}{2^{s-1} + 1}. \quad (9.2)$$

The remaining formulae of this section, apart from the trivial (3.22)–(3.24), show that for $? \times ?$, if $y = z = 0$,

$$P(M \times MN) = \frac{2^x}{(2^{x-1} + 1)^2}, \quad (9.3)$$

$$P(MN \times MN) = \frac{1}{(2^{x-1} + 1)^2}, \quad (9.4)$$

and if $x = z = 0$,

$$P(M \times MN) = \frac{1}{3 + 2^{y-1}}, \quad (9.5)$$

$$P(MN \times MN) = \frac{1}{3 + 2^{y-1}}, \quad (9.6)$$

$$P(N \times MN) = \frac{1}{3 + 2^{y-1}}. \quad (9.7)$$

For each of the matings $? \times M$, $MN \times ?$, and $? \times ?$ two-way tables may now be drawn up showing, for the case $z = 0$, the probabilities of the various complete matings; the results for $x = 0$ are inferred by symmetry. It has not been thought necessary to reproduce these tables here. By the use of them may be obtained the values of κ for different sets of x , y , and z , and these should next be tabulated. All the matings to be considered come under type 20a, in the nomenclature of this paper. The three cases $\delta^? \times \varphi M$, $\delta^? \times \varphi MN$, and $\delta^? \times \varphi N$ have, respectively $p_2 = p_3 = 0$, $p_1 = p_3 = 0$, and $p_1 = p_2 = 0$; thus the value of κ given in (4.17) simplifies very considerably. It will be found that, when the phenotypes of the children are

Table 1. *Values of κ for $\delta MN \times \varphi ?$ ($z = 0$ and $m = n = \frac{1}{2}$)*

$x \backslash y$	0	1	2	3	4	5	6	7	8
0	—	—	0.250	0.750	1.500	2.500	3.750	5.250	7.000
1	—	0.250	0.750	1.500	2.500	3.750	5.250	7.000	
2	1.000	1.889	3.222	5.000	7.222	9.889	13.000		
3	3.000	4.920	7.480	10.680	14.520	19.400			
4	6.000	9.160	13.111	17.852	23.383				
5	10.000	14.429	19.931	25.945					
6	15.000	20.642	27.224						
7	21.000	27.706							
8	28.000								

Table 2. *Values of κ for $\delta^? \times \varphi ?$ ($z = 0$ and $m = n = \frac{1}{2}$)*

$x \backslash y$	0	1	2	3	4	5	6	7	8
0	—	—	0.040	0.061	0.050	0.028	0.012	0.005	0.002
1	—	0.111	0.333	0.667	1.111	1.667	2.333	3.111	
2	0.111	0.680	1.160	1.800	2.600	3.560	4.680		
3	0.120	1.519	2.309	3.296	4.481	5.864			
4	0.074	2.567	3.675	5.003	6.554				
5	0.035	3.829	5.240	6.885					
6	0.014	5.320	7.017						
7	0.005	7.055							
8	0.002								

not such as to make the remaining p value unity, κ has the series of values occurring in the column $y = 0$ of Table 2, x for this purpose being interpreted as the total number of scorable children. The κ values for $\delta MN \times \varphi ?$ and $\delta^? \times \varphi ?$ with families of eight or less children are shown in Tables 1 and 2 for the case of $z = 0$; the case of $x = 0$ is symmetric on account of the equal frequencies of the M and N genes. The matings $\delta M \times \varphi ?$ and $\delta N \times \varphi ?$ can contribute no information.

For example, if $x = z = 0$, the expression of (4.17) reduces to

$$\kappa = \frac{1}{2}(p_1 + p_3)^2 y(y-1). \quad (9.8)$$

If $y = 5$ and the mating is $\delta MN \times \varphi ?$, the probabilities of $?$ being M , MN , or N are respectively, by the formulae of § 3, $\frac{1}{4}$, $\frac{1}{2}$, and $\frac{1}{4}$, and the value of κ is thus $\frac{1}{4}$. On the other hand,

for $\delta? \times \varphi?$, the probabilities are, by (9.5)–(9.7), $\frac{1}{36}$, $\frac{1}{18}$, and $\frac{1}{36}$, allowance being made for the fact that the father must be MN to be of interest. The information is then $\frac{10}{361}$ or 0.028.

In other cases of $z = 0$,

$$\kappa = \frac{1}{2}(p_1 + p_2)^2 x(x-1) + \frac{1}{2}p_1^2 \{2xy + y(y-1)\}. \quad (9.9)$$

For $x = 3$, $y = 2$, the probability that $\delta MN \times \varphi?$ is actually $\delta MN \times \varphi M$ is $\frac{4}{9}$ and that it is $\delta MN \times \varphi MN$ is $\frac{1}{9}$, $\delta MN \times \varphi N$ being in the circumstances impossible. Substitution in (9.9) leads to $\frac{187}{25}$ or 7.480 as the value of κ . With $\delta? \times \varphi?$ the corresponding probabilities are easily found as $\frac{4}{9}$, $\frac{1}{9}$, and 0; these lead to $\frac{187}{81}$ or 2.309 for the information. The full tables may readily be constructed from the constant second differences of the values for a given x .

It will be noted that the instructions of the present paper regarding the scoring of matings for which the paternal MN type is unknown and the maternal known differ slightly from those of § 5 of II. It is now recommended that the ancillary information given by the numbers x , y , and z should be fully employed for these. The effect of this is to replace the probabilities (5.19) and (5.22) of II by (3.19), (3.20), and (3.23) of this paper, the form of the observed information functions being unchanged. When this ancillary information is utilized, the expected values of κ do not differ from the observed. Details of the slight alterations in the scoring of these families will not be given here; it will suffice to state that the total score for the families listed in Table 7 of II is now

$$S(\lambda) = 1.599, \quad (9.10)$$

with information

$$S(\kappa) = 8.017. \quad (9.11)$$

There are eleven families in the records having $\delta MN \times \varphi?$ parentage. Their scoring need not be set out in full, but may be performed in the usual manner by a logical arrangement of the steps. For each family is tabulated in successive columns first the numbers of children of the six phenotypes and next the values of x , y , z . These are followed by columns for λ_1 , λ_2 , λ_3 , the scores corresponding to the three complete matings which could be of use for linkage, and others for p_1 , p_2 , p_3 , the estimated probabilities of these matings. Next the computation of λ is made, and the value of κ read from Table 1. The total score for these families is

$$S(\lambda) = 1.000, \quad (9.12)$$

with information

$$S(\kappa) = 4.500. \quad (9.13)$$

A similar tabulation, and the use of Table 2, enables the 137 families for which neither parent was tested for MN to be scored, with the result

$$S(\lambda) = 4.288 \quad (9.14)$$

and

$$S(\kappa) = 32.811. \quad (9.15)$$

Adding the information obtained in II from the families whose complete mating types were known, the result of the scoring of 200 families is found to be

$$S(\lambda) = 10.887 \quad (9.16)$$

and

$$S(\kappa) = 79.128. \quad (9.17)$$

The equation of estimation is therefore

$$1 - 4x = 0.138 \pm 0.112. \quad (9.18)$$

This estimate differs little from that given in II, but its precision is almost doubled.

The comparison begun previously of this efficient scoring of families with that obtained by the Penrose sib method may now be completed as in Table 3. For the 200 families the Penrose method has extracted only 20% of the available information. To obtain a test of the linkage by the method of sib-pairs as precise as that here obtained by efficient scoring about 1000 families would be required. Even if it is admitted that the computations for the efficient test are more laborious than those for the Penrose method, the extra labour is negligible compared with that of examining 800 additional families; the writer considers that with practice the difference in computation times for the two methods would be small.

It was surmised in II that the loss of information on this linkage test caused by inefficient scoring of the last group of 137 families would be comparatively small, but it is now seen that

Table 3. *Comparison of two forms of scoring for Sex \times MN linkage*

Parents tested for M, N	No. of families	Method of scoring			
		Penrose sib-pairs		Efficient λ	
		$(1 - 4x)$	Information	$(1 - 4x)$	Information
Both	29	0.161	6.340	0.118	34.000
Mother	23	0.426	2.444	0.199	8.017
Father	11	0.112	2.145	0.222	4.500
Neither	137	-0.014	4.878	0.131	32.611
Total	200	0.141	15.807	0.138	79.128

it is actually about 85 %. One family alone, family 126, having 2M, 1MN and 2N children, proving it to have both parents heterozygous, contributes 6 units of information, an amount greater than the total obtained by the sib-pair method. The remarks made previously must therefore be withdrawn, and it must be advised that, wherever possible, an efficient method of scoring should be used. In cases for which an efficient technique is not available, as for example when one or both parents are of unknown genotypes for both factors of a linkage test, the Penrose method allows the recovery of some information, but the proportion to be expected is at present uncertain. As was shown in II, scores by the two methods on different groups of families may be combined in order to make the best possible use of the available information and methods of analysis. It is probable here that the use of an empirical variance computed from the observed scores, as advocated in § 5, would bring about a substantial increase in information by comparison with the sib-pair method, but no examination of this point has yet been made.*

It is of interest to consider the loss in information which occurs when testing of parents

* Later investigations, soon to be published, confirm this view.

THE DETECTION OF LINKAGE

Table 4. *Alternative scorings of Sex \times MN linkage*

Family	x	y	z	λ_1	λ_2	λ_3	λ_1	κ_1	λ_{II}	κ_{II}	λ_{III}	κ_{III}	λ_{IV}	κ_{IV}
119	1	2	2	3	-1	6	-1	3	-1'000	3'000	-1'000	3'000	-1'000	3'000
120	—	—	2	0	-1	-1	-1	1	-0'333	0'111	-1'000	1'000	-0'333	0'111
88	2	—	—	1	1	0	1	1	0'333	0'111	1'000	1'000	0'333	0'111
89	—	2	—	1	0	1	0	0	0'333	0'111	0	0	0'200	0'040
132	—	2	2	1	1	6	6	6	6'000	6'000	4'333	3'222	2'600	1'160
1	—	6	2	-1	1	4	1	1	0'333	0'111	3'000	13'000	1'800	4'680
2	—	2	—	1	0	1	0	0	0'333	0'111	0	0	0'200	0'040
3	1	3	—	-2	0	-1	0	0	0	0	-1'000	1'500	-0'667	0'667
65	3	1	—	0	3	0	0	6	0	6'000	0'600	4'920	0'333	1'519
147	—	3	—	-1	0	-1	0	0	0	0	0	0	-0'143	0'061
148	2	—	—	1	1	1	1	1	0'333	0'111	1'000	1'000	0'333	0'111
139	—	2	2	1	1	6	0	0	0'333	0'111	0	0	2'600	1'160
140	—	5	—	-2	0	-2	0	0	-0'118	0'035	0	0	-0'105	0'028
14b	1	3	—	0	0	3	0	0	0	0	0	0	0	0'667
14c	—	2	—	-1	0	-1	0	0	-0'333	0'111	0	0	-0'200	0'040
15	4	—	—	-2	-2	0	0	0	-0'222	0'074	0	0	-0'222	0'074
12	—	3	—	-1	0	-1	0	0	-0'200	0'120	0	0	-0'143	0'061
13	2	1	1	-1	-1	-1	-1	3	-1'000	3'000	-1'000	3'000	-1'000	3'000
22	—	—	4	0	0	0	0	0	0'074	0'074	0	0	0	0'074
16	3	4	—	1	-1	0	0	0	-0'200	0'120	0	0	0'333	4'481
11	—	—	5	0	10	10	0	0	0'588	0'035	0	0	0'588	0'035
127	—	2	—	-1	0	-1	0	0	-0'333	0'111	0	0	-0'200	0'040
129	—	2	1	-1	0	-1	0	0	0	0	-0'500	0'750	-0'333	0'333
130	1	1	—	-1	0	0	-1	1	-1'000	1'000	-0'500	0'250	-0'333	0'111
131	1	1	—	1	0	0	0	0	0	0	0	0	0'333	0'111
149	4	1	—	-2	-2	0	-2	10	-2'000	10'000	-2'000	9'160	-1'059	2'567
150	—	1	1	0	0	1	1	1	1'000	1'000	0'500	0'250	0'333	0'111
123	—	2	—	-1	0	-1	0	0	0	0	-0'500	0'250	-0'200	0'040
144	—	3	—	3	0	3	0	0	0'600	0'120	0	0	0'429	0'061
Total	25	54	22	—	—	—	4	34	3'447	31'577	2'933	42'302	4'477	24'494

is incomplete. In the example of Sex \times MN linkage just discussed the thirty-four families having complete parental descriptions have been scored in four ways. These are (i) λ_1, κ_1 making use of the full descriptions as in II, (ii) $\lambda_{II}, \kappa_{II}$ obtained by considering the paternal MN types to be unknown, (iii) $\lambda_{III}, \kappa_{III}$ similarly obtained by ignoring the maternal MN types, and (iv) $\lambda_{IV}, \kappa_{IV}$ resulting from an assumption of ignorance of both parental MN types. The scores and informations pertaining to these four situations are set out in Table 4.

In practice such a scoring table as this would be begun with columns for the numbers of children of the different phenotypes, and would also contain entries for the probabilities to be attached to the basic scores, $\lambda_1, \lambda_2, \lambda_3$, for the three complete matings giving information on linkage, but, for considerations of space, these have been omitted here. The behaviour of family 1 is worthy of especial notice. The mating is $\delta MN \times \varphi MN$ and has $2\delta MN, 2\delta N$, and $4\varphi MN$ children; hence $x = 0, y = 6, z = 2$. Scored according to the actual parental types, and thus ignoring MN children, $\lambda_2 = 1$. The score based on M and MN children only is $\lambda_1 = -1$, but the existence of N children shows this to be of no interest; the score as $\frac{1}{2}u_{11}$ on MN and N children only, appropriate to the mating $\delta MN \times \varphi N$, is $\lambda_3 = 4$. If the MN group of the father were unknown, the probability of its actually being MN is assessed as $p_2 = \frac{1}{3}$ by the use of (3.19), both p_1 and p_3 being zero. The score, $p_2\lambda_2$, is then 0.333 and the information, obtained from (4.17) which has been said to be the value for mating type 20a, is 0.111. The same κ is obtained by entering Table 2 with $y = 0$ and $x = 2$, the number of scorable children. On the other hand, if the maternal MN group were unknown, as before $p_2 = \frac{1}{3}$, but the possibility that the complete mating is $\delta MN \times \varphi N$ must also be considered, and this has $p_3 = \frac{2}{3}$. This latter mating would be much more informative on linkage, since its scoring would be based on the eight children; (4.17) shows that

$$\kappa = \frac{1}{2}[\frac{1}{6} \times 2 + \frac{4}{6} \times 56 + \frac{4}{6} \times 2] = 13,$$

as may be read from Table 1. These two matings again are the possibilities to be considered under conditions (iv) when both parental blood groups are unknown, but the probabilities are now obtained from (3.25) and (3.26) and are $p_1 = 0, p_2 = \frac{1}{6}, p_3 = \frac{5}{6}$. The score is then

$$\lambda = \frac{1}{6} \times 1 + \frac{5}{6} \times 4 = 1.8,$$

and κ from Table 2 is 4.680.

This large family of rather abnormal constitution unfortunately somewhat upsets the comparison of the four situations with regard to parental descriptions listed above. But, even making allowance for the large contribution from this family to κ_{III} and κ_{IV} , there is evidence from the totals of the columns that the loss of information on this linkage test resulting from the loss of one parent is small. Indeed, when both parents are missing from the records the total loss of information is only of the order of 25 %. The four total scores in Table 4 are in good agreement. This suggestion that partial absence of parental descriptions may not entail great loss of information is supported by scoring those families with only one parent tested for MN type as though neither parent was available. If that one parent is the mother, the value of $S(\kappa)$ is increased from 8.017 to 9.245; similarly for the

father, on eleven families only, there is an increase from 4.500 to 7.712. These observed *increases* in information can only be the result of sampling variations and the ascertainment of a few large families of abnormal character; they must not be taken as indicating any real advantage of having less detailed parental descriptions! For the case of a sex linkage, however, there is the suggestion that no great loss of information will necessarily occur from such incomplete descriptions. On the other hand, for an autosomal linkage, failure to test one or both parents probably results in incomplete knowledge of parental phenotypes in respect of both genetic factors. This situation is likely to be much more serious, as not only may it cause a greater decrease in the information contained in a family, but at present it is only possible to recover this information efficiently by empirical means.

Further examination of this point may be made by a detailed study of the information arising from various types of family for this linkage test. Thus when both parents have their *MN* groups known it will be expected that of all families the proportions of the three types giving information on linkage will be $\frac{1}{8}$, $\frac{1}{4}$, and $\frac{1}{8}$. For families of two children $\delta MN \times \varphi M$ and $\delta MN \times \varphi N$ matings will give 1 unit of information; $\delta MN \times \varphi MN$ matings will give 1 unit in the $\frac{1}{4}$ of cases for which neither child is *MN*, and otherwise zero. The average information per family is therefore $\frac{5}{16}$. Similar analysis for families of which only the mother has been tested shows there to be an average of $\frac{5}{24}$ units of information. For families of two children of which only the father was tested the average is $\frac{7}{32}$ and for the case of neither parent known the average is $\frac{7}{80}$. The expected amount of information from families of two recovered by the Penrose method of sib-pairs can be found by the formulae of II, and averages $\frac{1}{4}$ per family. These results are compared in Table 5 with the observed quantities from all families of two in Boyd's records when these are scored in the appropriate manner.

Table 5. *Observed and expected information per family from families of two, in the Sex \times MN linkage test*

Type of scoring	Number of families	Information per family	
		Observed	Expected
Both parents tested	11	0.455	0.312
Father not tested	27	0.235	0.208
Mother not tested	17	0.397	0.219
Neither parent tested	134	0.108	0.117
Sib-pairs	134	0.023	0.042

This table shows the loss of information resulting from incompleteness of parental description, and the further loss from inefficient scoring. Even when neither parent is described the method of sib-pairs may be expected to recover only about one-third of the available information from families of two. Larger families should in general leave parental genotypes in less doubt and thus the proportionate increase in information to be expected from fuller testing of the parents should be less.

10. SUMMARY

When the parental phenotypes of families in human pedigree collections are, completely or in part, unrecorded, it is possible to assess the probabilities of different combinations from the observed numbers of children of different phenotypes, and formulae for these probabilities in terms of the population gene frequencies are given in § 3. The efficient score for linkage purposes of any such family is then simply the sum of scores for every possible mating, each being multiplied by its probability.

In later sections expressions are given for the information available, in families from the mating types discussed in I, when the incompleteness of parental testing is in respect of one factor only. In spite of the apparent complexity of these expressions, the labour involved in their use will generally be repaid by the increased information obtained. Information functions have not yet been developed for cases in which the descriptions of the parents are incomplete for both factors, but for any particular body of data an empirical value may be obtained from the observed variance of the scores, as has been indicated in § 5. This is an adaptation of a suggestion due to Fisher, but it seems preferable, and equally simple computationally, to use the fully efficient scores rather than the 'intermediate' type which he introduced.

An illustration of the methods of this paper is taken from data recently published by Boyd, and concerns a test of the possibility of a partial sex-linkage of the *MN* blood groups. A fivefold increase in precision is obtained by efficient scoring of 200 families by comparison with the test made by Boyd, but there is still little indication that a linkage may exist. It is shown that, both theoretically and in practice, there is a considerable increase in the information per family even from that section of the records which consists of sib-ships only.

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THE RELATIONSHIP OF PLANT NUMBER AND YIELD IN SUGAR-BEET AND MANGOLDS

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1. *Introduction*

DURING the last two years an extensive survey of wireworm infestation and the consequent damage to crops has been undertaken by the Ministry of Agriculture Plant Pathological Laboratory and provincial Advisory Entomologists, in consultation with Rothamsted Experimental Station. In the present season estimates of wireworm population have been made by a sampling process for about 400 fields, and wherever possible these have been followed by further sampling for plant density at an early stage of growth and later for yield. The results of this investigation are not yet complete, but it is clear that one important aspect of the problem is the extent to which plant elimination, by wireworm attack early in the growing-period, can be taken as an indication of the ultimate effect on yield.

Available evidence on the effect on yield of a reduction in plant density should throw some light on this point. Within the limits of normal agricultural practice, any elimination of plants will allow neighbouring plants greater freedom of growth. The increased size of these remaining plants will, to some extent, compensate for their reduced number and the loss in yield will thus be proportionately less than the loss in stand. With the object of discovering the extent of this compensation, experimental data from a number of sources have been examined, and the results of this examination are described below.

2. *Factory Sugar-beet Series*

For each of 52 experiments in the Factory Sugar-beet Series of the years 1933-9 [1] the error regression of yield on plant number has been obtained, these experiments being mostly those for which plant number showed great variability from plot to plot. The mean plant number (1,000 per acre) was 25.75 and the mean yield of sugar (cwt. per acre) was 33.75. Thus the mean weight of sugar from 1,000 plants was 1.311 cwt.

The regression coefficient of the 52 yields on the corresponding plant numbers was 0.647 ± 0.356 ; thus, from experiment to experiment, a decrease of 1,000 plants was accompanied by a decrease of 0.65 cwt. of sugar or 49.4 per cent. of the mean yield of 1,000 plants.

A similar percentage may be calculated for each experiment separately, from the regression coefficient within the experiment. This quantity is a measure of the independence of growth of (absence of competition between) the roots. Clearly a value of zero means that changes in plant density had no effect on yield, and thus there was complete compensation for any decrease in density by an increase in

weight per plant. On the other hand, a value of 100 per cent. means that competition was so completely absent that a decrease in plant number had no associated compensation of increased weight per plant. The mean value of the percentage (k) for the 52 experiments was 53.4, which agrees very closely with the corresponding figure above calculated between experiments. There was no evidence of any association between the values of k and the mean plant densities of the experiments, but there was, as might reasonably be expected, an increase in k with decreased yields. If s represents the yield of sugar in cwt. per acre, the regression equation of k on s is

$$(k - 53.4) = -1.62(s - 25.8),$$

the standard error of the regression coefficient being ± 0.77 .

This evidence suggests that for a normal yielding crop, and within reasonable limits of plant density, any loss of plants will result in a loss in yield of about 50 per cent. of that which would occur if the remaining plants gave no compensatory increase in yield per plant. The heavier the yield, the greater will be the degree of compensation and consequently the less will be this proportionate loss, the estimated percentage decreasing by 1.6 for every 1 cwt. increase in total sugar per acre.

Of the 52 values of k , 12 are actually greater than 100 per cent., implying an increase in yield for each additional plant greater than the mean weight of a plant. It is likely that some over-estimation of the regression coefficient of yield on plant number occurs in some experiments, because of a correlation between plant number and plot fertility, such that plots with a high plant number yield more *per plant* than those with a lower plant number receiving the same treatment. This would result in an over-estimation of the values of k . It is thus possible that the true reduction in yield for a decrease in stand is less than the estimated value of 50 per cent.

3. *The Influence of Gaps*

In 1926 an experiment to test the effect on yield of a plant population reduced below a full stand by gaps was performed by E. Lindhard and M. Jørgensen of the Royal (Danish) Agricultural College [2]. Two varieties of mangold (Sludstrup Barres, a large-topped variety, and Taarøje Barres, a small-topped variety) were tested at Lyngby and sugar-beet (Kleinwanzleben N) was grown at the College farm. At each centre the seed was sown at a drill width of 60 cm., the plants being later thinned to exactly 25 cm., to give a plant density of 66,700 per hectare (27,000 per acre). The experimental area had 19 rows at least 50 m. in length.

Between 8 and 14 days after thinning, artificial gappiness was introduced by removing from every fourth row runs of 1, 2, 3, or 4 successive plants, 6 plants being left standing between gaps. In any given row all gaps were of the same length. These rows are referred to as 'gap rows'; of the three rows between successive gap rows, the outer two are 'neighbour rows' and the middle one a 'normal row'. The remaining plants of a gap row may be classified as 'gap roots' (immediately adjacent to

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the gaps) and 'normal roots'. The runs of 1, 2, 3, or 4 plants in a neighbour row parallel and adjacent to a gap are called 'neighbour roots'; other plants of the same row, including those diagonally adjacent to a gap, are again called 'normal roots'. There were 20 replicates of each type of gap.

For each gap row, separate determinations of yield and dry matter per root were made for gap roots and normal roots. Separate figures were also obtained from the corresponding neighbour rows for neighbour roots and normal roots. The results are shown in Table 1 (Table 7 of [2]).

TABLE 1. *Weight of Normal, Gap, and Neighbour Roots for Gaps of Different Size*

Row	Root	Weight in gm. per root									
		Total					Dry matter				
		1 gap	2 gaps	3 gaps	4 gaps	Normal mean	1 gap	2 gaps	3 gaps	4 gaps	Normal mean
Sugar-beet (Landbohøjskole)											
Gap	Normal	662	680	650	642	658	156	159	153	151	155
Neighbour	Gap	935	1,040	1,113	1,070	..	213	234	249	240	..
	Normal	651	660	657	640	652	153	155	154	151	153
	Neighbour	667	696	737	732	..	157	163	172	170	..
Mangolds—Sludstrup (Lyngby)											
Gap	Normal	1,083	1,092	1,062	1,085	1,080	131	132	129	132	131
Neighbour	Gap	1,406	1,623	1,613	1,790	..	165	186	185	203	..
	Normal	1,159	1,172	1,149	1,193	1,158	139	140	138	143	140
	Neighbour	1,272	1,234	1,252	1,333	..	151	147	149	157	..
Mangolds—Taareje (Lyngby)											
Gap	Normal	1,244	1,278	1,331	1,344	1,299	132	135	140	141	137
Neighbour	Gap	1,681	1,856	1,877	2,165	..	171	186	188	213	..
	Normal	1,342	1,330	1,372	1,351	1,349	140	139	143	141	141
	Neighbour	1,227	1,381	1,455	1,464	..	130	144	151	152	..

From this table it is possible to estimate the actual loss in yield caused by a gap of given size after allowing for the compensatory increased growth of adjacent plants. Thus, from the sugar-beet data in the 4-gap column, the expected yield from the two gap roots and eight neighbour roots is

$$2 \times 1070 + 8 \times 732 = 7996 \text{ gm.}$$

Had there been no gaps, the expected yield from roots in the corresponding positions would be

$$6 \times 642 + 8 \times 640 = 8972 \text{ gm.}$$

The total loss for the gap is 976 gm., or 1.52 times the weight of a normal root in the same rows (641 gm.). These estimated losses are shown in Table 2 (which is part of Table 9 of [2]).

Table 1 shows there to have been little difference between the weights of normal roots in gap and in neighbour rows. The increased weight of

the two gap roots is very marked for all three varieties of root; the difference from normal increases with the length of gap, the rate of increase for mangolds showing little sign of falling off even for a 4-gap, though for sugar-beet there appears to be no effect on these roots of increasing the gap beyond 3 root-places. Neighbour roots, on account of their greater distance, cannot so readily take advantage of the gap and their increases in weight are comparatively small, though again the effect increases steadily with increasing gap size.

From an examination of 800 plots, Pedersen [3] has concluded that the distribution of gaps of different sizes is substantially that which would be expected if the elimination of plants took place entirely at random. In a further paper [4] the same author applied this result to the

TABLE 2. *Loss in Yield for Gaps of Given Size, in Units of the Weight of One Normal Root*

	Total				Dry matter			
	1 gap	2 gaps	3 gaps	4 gaps	1 gap	2 gaps	3 gaps	4 gaps
Sugar-beet	0.13	0.72	0.85	1.52	0.22	0.86	1.08	1.78
Mangold (Sludstrup)	0.21	0.82	1.42	1.76	0.32	0.99	1.67	2.11
Mangold (Taarøje)	0.47	0.95	1.82	2.11	0.55	1.10	1.99	2.39
Mean	0.27	0.83	1.37	1.80	0.36	0.99	1.58	2.09

estimation of the loss in yield resulting from a given percentage reduction in stand. For any such percentage the proportions of gaps of different length caused by plant elimination was estimated according to the law of randomness and the appropriate loss in yield for gaps of each length was then obtained from Lindhard and Jørgensen's experimental data. Unfortunately these data gave no information on gaps of more than 4 root-places, and it was therefore necessary to obtain the required figures by extrapolation of a smooth curve. Such a method is clearly open to objections, but its effects on Pedersen's conclusions can scarcely be very great below 50 per cent. plant elimination, since below that figure few large gaps will occur. A further point which should be noted is that no allowance is made for non-independence of gaps. With a large percentage loss in stand the gaps will seldom be separated by six plants, as in Lindhard and Jørgensen's experiment, and not infrequently gap roots will occur which have gaps on either side. Gaps may also occur side by side in adjacent rows. Such situations will presumably lead to underestimation of the loss in yield.

Pedersen's tables may conveniently be expressed in terms of the coefficient k , previously defined as the loss in yield for a reduced plant density as a percentage of the normal yield of the number of plants eliminated. Indeed, Table 8 of [4] gives the values of this coefficient for yields of dry matter, the two varieties of mangold having been averaged; Table 3 below is extended so as to cover total root-weight also.

The percentage k is the complement of e , the coefficient of utilization defined by Pedersen (i.e. $k = 100 - e$). For both crops it is noticeable

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that the values are about 8 per cent. higher for dry matter than for total weight, showing that the compensatory increases in weight of plants which benefit from empty root-places are made at the expense of a decreased proportional dry-matter content. The figures for dry matter

TABLE 3. *Values of k, the Loss in Yield as a Percentage of Normal Yield of Missing Plants*

Percentage of empty root-places	Sugar-beet		Mangolds	
	Total	Dry matter	Total	Dry matter
5	17	26	35	45
10	18	27	37	46
20	23	31	40	49
30	27	35	43	52
40	33	40	47	55
50	39	46	52	59
60	47	53	57	64
70	56	61	64	70
80	68	71	73	77

in Table 3 are fairly satisfactorily in agreement with the average value of 50 per cent. found for the sugar-beet series examined earlier, though the coefficient there showed no association with plant density.

4. *Experiments in Denmark*

Pedersen concluded his second paper with an examination of the results of 2,300 experiments on mangolds, conducted by the Danish Agricultural Organizations in Sjælland, Fyn, and Jylland in the period 1926-30. In Table II of the paper he shows these experiments grouped according to their plant density, the mean yield for each group being given. The results for Sjælland and Fyn are very similar and for the present purpose have been combined; in Jylland both yields and plant densities were lower. The experiments are summarized in Table 4.

TABLE 4. *Results of Danish Mangold Experiments, 1926-30*

	<i>Sjælland and Fyn</i>	<i>Jylland</i>
No. of experiments	1,416	886
Mean yield (hkg./ha.)	748	603
Mean plant density (1,000/ha.)	63.2	51.7
Mean yield per 1,000 plants (hkg.)	11.8	11.7
Decrease in yield per 1,000 plants reduction (hkg.)	6.56	6.05
k (per cent. proportional decrease)	55.6	51.7

Fig. 1 shows, for each set of experiments, the straight line and parabola which best represent the yields in terms of plant number. Measuring plant density (x), in 1,000 per hectare and yield (y) in hectokilograms per hectare, the line and parabola for Sjælland and Fyn are

$$y = 344.6 + 6.39x,$$

and

$$y = 133.1 + 13.13x - 0.0520x^2.$$

The corresponding curves for Jylland are

$$y = 319.3 + 5.48x,$$

and

$$y = -43.5 + 19.54x - 0.1305x^2.$$

It is apparent that, for the first group of experiments, the straight line is almost as good a representation of the results as is the parabola, but

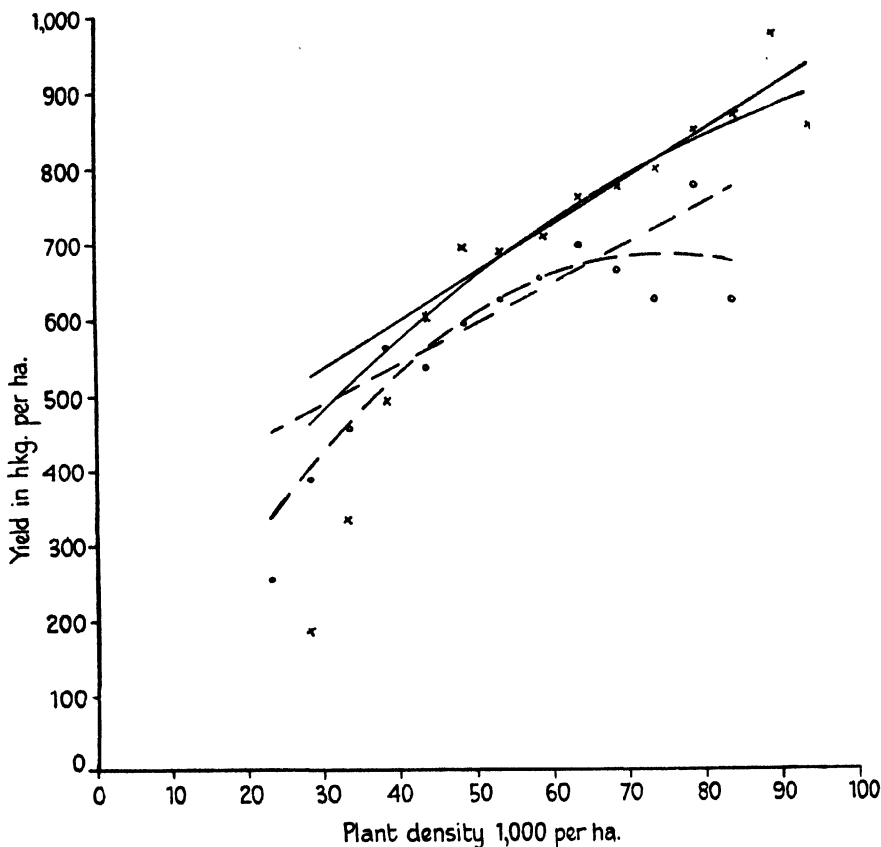


Fig. 1. Plant density and yield in mangold experiments of the Danish Agricultural Organizations, 1926-30.

(The experiments were grouped according to plant density. The yields plotted are the means for densities of 21,000-25,000, 26,000-30,000, . . . plants per ha.)

× and ——— Sjælland and Fyn (1,416 experiments)
○ and ——— Jylland (886 experiments).

for the Jylland experiments the parabola is successful in showing the much reduced response of yield to increasing plant density when the latter exceeds 50,000 per ha. Indeed, there are indications here that the yield may actually be reduced when the density exceeds 80,000 per ha. In passing it may be observed that, though these parabolas have

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not been constrained to show zero yield for $x = 0$, their continuations do in fact pass reasonably close to the origin.

By differentiation of the equations of the parabolas, the decrease in yield for a loss of 1,000 plants per ha. corresponding to any given plant density may be found. Taking the mean density for each series of experiments, it may be shown that with a plant population of 63,200 per ha. in Sjælland and Fyn, a decrease of 1,000 plants per ha. might be expected to produce a decrease in yield of 6.56 hkg. per ha.; the corresponding decrease for a population of 51,700 per ha. in Jylland is 6.05 hkg. per ha. Expressing these decreases as percentages of the mean yield per 1,000 plants, the values of the coefficient k are found to be 55.6 and 51.7 per cent., figures which show remarkably close agreement with each other and with those obtained in the first section of this paper.

5. Experiments in Holland

In 1930 and 1931 experiments on the weight of individual beet were conducted at the Sugar-beet Institute, Bergen-op-Zoom, Holland [5, 6]. On three plots in the first year, and on one plot in the second, each root was weighed separately and its distance from the two adjacent roots in the same row was measured. The mean distance was multiplied by the width between rows to give the area occupied by the individual root and

TABLE 5. *Results of Dutch Sugar-beet Experiments, 1930-1*

	1930			1931
	Plot A	Plot B	Plot C	
No. of beet	465	939	895	3,204
Mean yield (hkg./ha.)	489	555	530	356
Mean plant density (1,000/ha.)	55.7	59.1	61.5	54.8
Mean yield per 1,000 plants (hkg.)	8.8	9.4	8.6	6.5
Decrease in yield per 1,000 plants reduction (hkg.)	4.11	4.22	3.93	3.99
k (per cent. proportional decrease)	46.8	44.8	45.6	61.6

the mean root-weight in successive ranges of areas was calculated. These figures were then converted to show the number of beet per ha. and the corresponding mean yield in kg. per ha.

These results can scarcely be considered comparable with those discussed earlier. The growth of adjacent roots will be far from independent and competition-effects complex. By contrast, the other small-scale experiments were so designed as to permit the examination in some detail of the competition between adjacent roots at various distances. In the large-scale experiments, on the other hand, only average competition-effects for a plot or for a field were under discussion. Nevertheless there is some interest in treating the yield and plant-density figures in the same way as the Danish large-scale experiments. Table 5 summarizes the results.

As with the Danish data, the straight lines and parabolas best representing the yield-plant-density relationship were calculated. In all cases there is an appreciable curvature, and in 1931, the higher yielding year, there is a suggestion of a maximal yield at about 80,000 plants per

ha. In the same manner as previously, the expected decreases in yield for a reduction of 1,000 plants per ha. below the mean density were calculated and expressed as percentages of the mean yields of those plants. The values of k thus obtained were lower in 1930 and higher in 1931 than for the experiments previously discussed. Even so, none of the figures departs far from 50 per cent., and thus all show fair agreement with the British and Danish series.

6. Summary

Examination of the relationship between plant density and yield in four widely different series of experiments on sugar-beet and mangolds indicates that, with a normal plant population, any elimination of roots may be expected to be accompanied by about half the proportionate loss in yield. The values obtained for the proportion of the normal yield of the eliminated roots which is lost, a coefficient representing the degree of absence of competition, range only from 44.8 to 61.4 per cent. This range becomes even narrower (49.4 to 55.6 per cent.) if the Dutch experiments, which are not strictly comparable with the others, are omitted. With stands of greater than average density, the Danish and Dutch experiments agree in showing increasing competition, and thus a reduction in the expected loss from plant elimination; in the British series of experiments no such tendency was evident. It is possible that in the British and Danish large-scale experiments (and to a lesser extent, in the Dutch data), association between reduction in stand and the inherent fertility of the experimental sites may have led to over-estimation of the loss in yield; in the Danish small-scale experiments this can scarcely have occurred since stand was there artificially controlled.

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WIREWORM POPULATIONS AND THEIR EFFECT ON CROPS

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(With 1 Text-figure)

1. INTRODUCTION

THE objects of the scheme of investigations on wireworms, begun in 1939 by the Advisory Entomologists of the thirteen Agricultural Provinces of England and Wales, in co-operation with the Ministry of Agriculture's Plant Pathological Laboratory and the Statistical Department of Rothamsted Experimental Station, have been stated in the Interim Report (Anon. 1940) as:

'(a) To find a method of estimating the wireworm population of a grass field with sufficient accuracy and sufficient speed to be of service in advisory work.

'(b) To discover any obvious correlations between the size of the wireworm population in any field and other factors such as soil type or cultural treatment.

'(c) To obtain some guide as to the damage to be expected from wireworm populations of different sizes.'

The 100 fields of grassland about to be ploughed, which were examined during the first season, provided information on (a), on the basis of which improvements in the sampling technique were recommended, but the records were neither in numbers nor in content sufficient to do more. In consequence, an extended scheme of observations was designed for the Wireworm Survey of the 1939-40 harvest year. In addition to the estimation of infestation, and to estimation also of plant density and yield of the first crop after grass, this scheme embraced the recording of many other details relating to the history and character of the field and the crop. With these last it is not proposed to treat here, though they are clearly the data relevant to (b) above.

The first part of this paper, §§ 2-5, contains a discussion of (a) and of issues arising therefrom, making use of the records of nearly 500 fields. The information on plant population and on yield enables an investigation of points raised by (c) also to be made, and this comprises §§ 6-8. Unfortunately, the evidence available for this study is only sufficiently full for oats, and the discussion of other crops must await a further extension in the number of records. The term 'wireworm' as used in this report generally refers to the three common species of *Agriotes*, with *A. obscurus* predominating; occasional specimens of *Athous haemorrhoidalis* and of other species have been found and included in the counts without comment, their number being too few to have any bearing on the results.

2. THE SAMPLING TECHNIQUE

For the wireworm sampling undertaken during 1939 the standard size of sample was a 6 in. square of soil; samples were to be taken to the greatest depth at which wireworms could be found, for which purpose most observers seem to have been satisfied with a depth

of 6-9 in. As a result of these preliminary investigations, it appeared safe to recommend that in future a cylindrical core of 4 in. diameter should be used as the standard sample. If the same number of samples is taken there is only one-third of the volume of soil to be examined, a gain which outweighs the loss of information due to the lesser accuracy of the smaller samples. Equal precision in the estimation of populations will require approximately twice as many of the small samples as of the large. The area of a 4 in. core is almost exactly one five-hundred-thousandth of an acre, and thus the conversion of sample counts to estimated populations per acre is very simple.

It was advised that twenty samples per field should be taken, two being located randomly in each tenth of the field. This plan was chosen so as to allow a comparison to be made of the variation between and within the ten sections of the field. This number of samples was actually taken from most of the fields sampled, but in some cases only ten were taken, and instances occurred of other numbers up to a total of twenty-six samples. The arrangement of the samples in pairs was not always followed, a random selection of sampling points over the whole fields being sometimes used. Some observers in 1940 still preferred to use the 6 in. square as the sampling unit, and cases occurred of individual fields being sampled with 8 in. squares or with 3 in. cores, but the 4 in. core was the unit generally adopted.

It is recognized that the figures for the numbers of wireworms per acre used in subsequent sections do not represent the total populations present. The technique adopted for counting had necessarily to be sufficiently rapid to be of use in advisory work and it is known that the smaller larvae (below 8 mm. in length) were not exhaustively counted. Though these probably form a large part of the total population, it is evident from the results discussed in §§ 6 and 7 that the numbers of the larger wireworms, which are considered to have been almost fully ascertained by the technique used, were a good indication of the crop damage to be expected in the harvest year after sampling.

Records of samplings from 473 fields were received during the 1940 season. Most of the sampling took place before ploughing, either in the autumn of 1939 or in the late spring of 1940, and the few records from fields sampled after ploughing, either before or after sowing, can scarcely bias the general conclusions.

3. GEOGRAPHICAL DISTRIBUTION OF WIREWORM POPULATIONS

From an examination of the mean populations of the 473 fields sampled, there can be little doubt that wireworm infestation is considerably higher in the south of the country than in the north. The means for the thirteen Provinces are set out in Table 1, together with the percentage of fields having populations of more than 300,000/acre, a figure chosen as being one below which little damage to oats and wheat was found.

Differences between provinces might in part be the result of the conditions of counting the samples rather than of true differences in infestation. Examination by hand must inevitably introduce a personal element and the difficulties of discovering the smaller wireworms in a sample will be much greater in heavy than in light soil. Also it is known that some observers examine their samples in the field and others prefer to make their examination in the laboratory. Moreover, the fields sampled in any province are not necessarily fully representative of that province; in some instances almost all the fields were from a single county. Nevertheless the geographical trends in mean population appear too regular

to have been the chance result of any of these causes. The provinces have been grouped into three regions, south, west, and north, there being a distinct break in the sequence of mean populations between the lowest province of one region and the highest of the next. It is arguable that, in view of their exceptionally high infestations, the south-eastern and southern provinces should constitute a separate region, but, for the study of the relationship of infestation and crop there were too few fields available for this separation to be profitable. More extensive information on these differences should be obtained from the sampling of the 1940-1 season, as a result of which it should be possible to examine in greater detail the grouping of provinces, or even of counties.

TABLE 1. *Wireworm infestation in the thirteen provinces*

Province	No. of fields	Mean population in 1000/acre	% fields with more than 300,000/acre
South-eastern	82	1025	93
Southern	33	824	88
Eastern	50	627	72
Midland	42	540	62
Western	16	520	62
West midland	38	505	53
South Wales	29	394	59
South-western	35	327	43
Mid-Wales	17	308	24
Yorkshire	30	234	30
North Wales	30	225	27
North-western	40	208	8
Northern	31	133	10
South (total)	261	738	75
West (")	81	347	44
North (")	131	200	18
All	473	522	54

4. PATCHINESS WITHIN THE FIELD

It is sometimes argued that patchy distribution of wireworms within a field invalidates the use of an average figure for the population of the field. Cases are instanced of fields which are heavily infested at one end and almost free from wireworms at the other, for which any cropping recommendation based on the average population may have unfortunate results. Without prejudice to the use of more detailed recommendations in such cases, the general applicability of this criticism to all wireworm sampling may be tested by the comparison of the variability of populations between and within sections of the same field.

After rejection of fields with less than sixteen samples in order to ensure satisfactory estimation of the variabilities there remained 396 fields for which this comparison could be made. An analysis of variance for 'between' and 'within' sections was carried out on the counts from each field. The fields were then classified into three groups according to the ratio of the mean squares, the ratios separating the groups being taken as 1.5 and 3.0 (see Table 2). This ratio may be taken as a measure of the patchiness of a field, since it shows the extent to which variation between sections exceed intrasection variability. If the distribution of wireworms in a field were purely random, it would be expected that 27 % of all fields would be sufficiently patchy to show a variance ratio of at least 1.5, and 5 % of all fields would have a ratio greater than 3.0.

There is clearly no sign of any excess of fields above the number expected in the middle category, but, for the south and west regions only, about 12 % of all fields are found in the group of greatest patchiness instead of the 5 % expected. There is no evidence of any departure from randomness in the north. It seems then, that in the more heavily infested districts, there is some tendency for the larvae to be congregated in patches instead of randomly distributed over the field. This effect is however quite small, and can scarcely be of much practical use or importance; certainly it does not invalidate the general use of the mean population as the measure of infestation of a field.

Indeed for any use to be made in practice of intra-field variation in infestation, it would presumably be necessary for there to be areas of a field sufficiently different in population and suitable in shape for them to be cropped separately. Examination of the location on the field of the samples from patchy fields suggests that less than one-fifth of such fields fulfil these two conditions. It thus seems probable that only about 5 % of all fields have sufficiently regular and pronounced trends in their degree of infestation to make the dividing of the field for cropping worth consideration.

TABLE 2. *Patchiness of infestation*

Region	No. of fields with variance ratio			Total
	—1·5	1·5–3·0	3·0–	
South	141	40	24	205
West	51	15	11	77
North	90	16	8	114
All	282	71	43	396

Though this analysis has vindicated the use in general of a mean population, it should not be taken to mean that in no circumstances need the distribution of infestation within the field be considered. Undoubtedly cases do occur of very great differences in population between the two sides of a field, and it is always open to the adviser to make his recommendations accordingly, if necessary taking additional wireworm samples from each division of the field in order to form satisfactory estimates of the separate populations. A cause of patchy failure of a crop which is possibly more important than the occurrence of areas of high wireworm concentration is the greater effectiveness of wireworm damage in destroying the crop in areas where the young plant has also to combat poor fertility conditions. Such a situation is very likely to arise on land newly brought under the plough, and for it the wireworm can scarcely be held directly responsible.

5. ESTIMATION OF SAMPLING ERRORS

Using the ordinary statistical technique, a standard error per sample was computed for each field. This was taken from the total variation of all samples and not from the 'within sections' mean square, as, in view of the evidence described in the preceding section, the former seemed in general to be the best available estimate. Considering for the present only those fields sampled by 4 in. cores, and rejecting a few on account of insufficient sampling, there remain 309 fields for the study of the sampling errors and their relation to the mean infestation.

When the average number of larvae per sample was small (less than 0·5, corresponding

to 250,000/acre) the distribution of the number found per sample was satisfactorily fitted by the Poisson Law; that is to say the sampling variance was equal to the mean per sample. For denser populations, however, the variance was higher than would result from the Poisson distribution. For each of the 309 fields the percentage standard deviation (coefficient of variation) was calculated and plotted against the mean count per sample. Though the points thus obtained were very variable, they indicated a decrease with increasing population which was less rapid than the Poisson Law would predict; when the mean count becomes high—of the order of 4.0—the percentage standard deviation is decreasing very slowly and is almost constant at about 80 %. The mean values of the ratio in successive small ranges of the mean count were computed and a smooth curve was drawn by eye through the points obtained. This curve, hereafter taken as representing the relationship between mean count and proportionate sampling error, is shown in Fig. 1. In this figure the curve for a Poisson distribution is also drawn, but the individual observations have not been shown, as the points are in many places very congested.

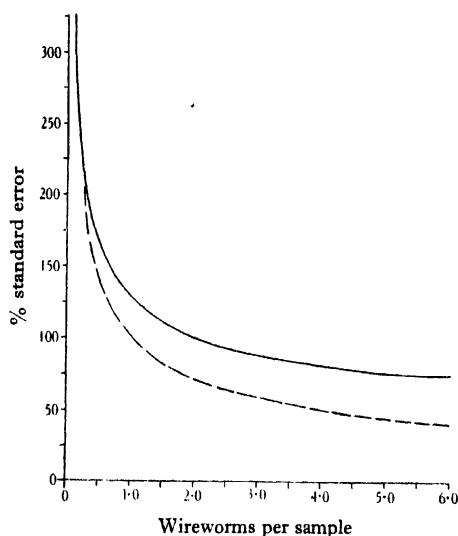


Fig. 1. Relationship between numbers of wireworms per sample and the sampling errors for a 4 in. core of soil. ——— Estimated from 309 fields. - - - Poisson distribution.

From the curve the sampling error corresponding to any population may be read. For example a population of 500,000/acre, averaging 1.0 larva per sample, has a sampling error of 126 % or 630,000/acre. The standard error of a mean population calculated from twenty samples is therefore 141,000/acre. Using a table of normal deviates, limits may be determined beyond which an observed mean of twenty samples will lie with a given probability. Thus the normal deviate for a 12.5 % probability is 1.150 and deviations of 162,000/acre or more will occur with a probability of 12.5 % at each extreme. That is to say, of fields having true populations of 500,000/acre, one-eighth will, on account of sampling variation, appear to have populations less than 338,000 and one-eighth more than 662,000 as a result of a sampling by twenty 4 in. cores. A series of results of this type is given in Table 3.

An alternative, and in practice more useful, aspect of the same results is shown in Table 4. The precise meaning of this table of fiducial limits needs careful thought, but may be briefly described as follows. From Table 3, a field population of 300,000/acre will be estimated by sampling to be greater than 418,000 once in eight times; similarly a population of 400,000/acre will once in eight be estimated greater than 541,000. By a linear interpolation, it is found that a true population of 367,000 will be estimated at a figure greater than 500,000 by one-eighth of the determinations. A similar interpolation shows that a population of 706,000/acre

TABLE 3. *Distribution of sampling means for populations of given density sampled by twenty 4 in. cores (thousands/acre)*

Density of population	$\frac{1}{8}$ of determinations less than	$\frac{1}{4}$ of determinations less than	$\frac{1}{2}$ of determinations greater than	$\frac{3}{4}$ of determinations greater than
100	40	65	135	160
200	105	144	256	295
300	182	231	369	418
400	259	317	483	541
500	338	405	595	662
600	416	492	708	784
700	495	580	820	905
800	578	670	930	1022
900	659	759	1041	1141
1000	739	847	1153	1261

A density of 100,000/acre corresponds to a total of 4 per 20 samples.

TABLE 4. *Probable limits of error of population values obtained from sampling by twenty 4 in. cores (thousands/acre)*

Observed density	Population value which would give a density			
	as great or greater than that observed		as small or smaller than that observed	
	in $\frac{1}{8}$ of the determinations	in $\frac{1}{4}$ of the determinations	in $\frac{1}{4}$ of the determinations	in $\frac{1}{8}$ of the determinations
100	—	—	144	192
200	130	154	264	323
300	204	239	380	452
400	285	327	494	579
500	367	415	609	706
600	449	504	722	827
700	531	593	834	951
800	613	682	947	1073
900	696	773	1059	1195
1000	781	863	1171	1313

One wireworm per twenty samples corresponds to 25,000/acre.

will give a density as small or smaller than 500,000 in one-eighth of the determinations. These two populations, 367,000 and 706,000/acre, are the lower and upper 12.5 % fiducial limits to an observed density of 500,000/acre. Fiducial limits for a series of observed densities up to 1,000,000/acre are shown in Table 4.

Similar tables may easily be constructed for any other number of samples per field. Tables for other sizes of sampling unit are not directly calculable from Fig. 1, but the results of the sampling by 6 in. squares in 1939 were used to obtain corresponding tables for that size of unit. For low population densities, where the Poisson Law operates, 6 in. squares are of

the same efficiency as 4 in. cores from the point of view of the precision of the estimates obtained, but when the density exceeds 500,000/acre, one 6 in. square is approximately equal in value to two 4 in. cores. Thus to obtain precision by 4 in. cores equivalent to that from 6 in. squares only one-third to two-thirds of the volume of soil need be examined, and the smaller sampling unit is in general to be preferred.

About twenty samples per field appears to be a satisfactory number to take in order to differentiate with reasonable assurance between heavy, medium, and light infestations. It has, however, been suggested that in areas of the country where the general level of infestation is low, small variations in population may be important in their effects on the crop. As will be seen from the ensuing sections of this paper, the present survey provides little evidence in support of this view, but if it should ever be necessary to practice a finer differentiation between these populations having proportionately high sampling variation, many more samples per field will be required.

Also, the sampling technique has so far only been tested for grassland, either before or immediately after ploughing. Until the sampling variations of fields sampled after cropping in 1940 have been examined, it is impossible to judge whether the same accuracy of estimation of populations can be obtained from 20 samples. It might be expected, for example, that there would be greater irregularity of distribution in a field of stubble than in grass, and that consequently more samples per field would be needed for adequate estimation, but there is not yet sufficient evidence on this point.

6. PLANT DENSITY AND WIREWORM POPULATION

Observers were asked to obtain, for as many as possible of the cereal crops, at least one sample determination per field of the plant density at an early stage of growth. The sampling technique employed was a slight modification of one that had been used for some years in sampling for yield, details of which have been described by Cochran (1938). The sampling unit was 2 ft. length of four adjacent rows (or, for a broadcast crop, an area 2 ft. square) and samples were taken at 30 or 60 yd. intervals in two lines along the field. Counts were recorded of all plants in each of the four rows of every sample.

The only crop for which any large volume of information has been collected in 1940 is oats; unfortunately the decision to take plant counts was made rather too late for many counts to be made on fields of wheat. At least one count was made on each of 183 fields of oats, and on 117 of these a second count was made one to four weeks later. Such second counts were to be made as late as possible, having regard to the increasing difficulty of distinguishing individual plants. The sources of sampling variation have not been examined in very great detail, but the figures for 41 counts from a single province indicate the chief variation to be between the two lines of sampling and that no very great gain would result from counting more rows per sample or taking more samples per line. The absolute values of sampling errors appear to be higher in the north, where plant densities also are higher, but, for a field of average size sampled according to the instructions, and thus having about 16–20 samples taken, the sampling error of the density computed from the mean count of the two lines may in general be taken as 15 % of the estimated density.

In considering the influence of wireworm infestation on plant density, it was decided to make use of the first plant count, rather than the mean, for fields on which two were taken. It was felt that this convention would bring the figures nearer to a true comparability having

regard to the development of the crop than would the use of an average count. The regression of plant count on wireworm population was then computed separately for the fields of each province. In order to obtain regression coefficients with a reasonable degree of precision, provinces were grouped once more into the three regions of § 3. The results are shown in Table 5.

A first point to be noticed about this table is the very much lower mean plant density in the south as compared with the other two regions. One cause contributing to this was probably the use of lower seeding rates in this part of the country. For the fields sampled in this group of provinces, $4\text{--}4\frac{1}{2}$ bushels/acre was a normal seeding rate, whereas in Wales and the North of England seeding rates of 5–6 bushels/acre, and sometimes even higher, were employed. Only in the north was there sufficient variation in the rate of seeding for its effect to be judged, but 64 fields from this region show the results given in Table 6. The plant counts were, for the most part, taken soon after the appearance of the plants above ground, and, as would be expected at this stage, they showed considerable dependence on the amount of seed sown, the relationship being about linear.

TABLE 5. *Regression of plant density on wireworm population*

Region	No. of fields	Wireworm (1000/acre)	Plants (1000/acre)	Loss in plants per additional wireworm
South	63	710	920	$0\cdot34 \pm 0\cdot10$
West	48	366	1230	$0\cdot49 \pm 0\cdot19$
North	72	218	1310	$1\cdot22 \pm 0\cdot26$
All	183	426	1160	$0\cdot46 \pm 0\cdot09$

TABLE 6. *Influence of seeding rate on plant populations (north only)*

Seeding rate (bushels/acre)	< 5	5–5½	> 5½
No. of fields	9	38	17
Plants (1000/acre)	1050	1270	1560

Undoubtedly the chief interest of Table 5 lies in its clear indication of a progressive increase from south to north in the plant loss per additional wireworm. The most obvious explanation of this phenomenon is that when the wireworm population is low most of the plants killed are attacked by one wireworm only, but as the infestation is increased the chance of a wireworm finding an untouched plant is lessened and many plants will actually be attacked by two or more wireworms, thus reducing the average rate of plant damage per wireworm. The true relationship between infestation and plant density should therefore not be linear; this view is supported by plotting the two figures for each of the 183 fields. Inspection of the diagram gives no indication of consistent differences between regions, but only of a steady falling off in the linear regression with increasing infestation. A further test of this explanation was provided by a more detailed examination of the 63 fields in the south, which were subdivided into those with wireworm populations above and below 500,000/acre.

Though the precision of the regression coefficients in the two groups is less than those previously considered, the numbers of fields involved being small, it is noteworthy that the results for fields with less than 500,000 wireworms/acre agree very closely with those for the north region. In contrast, the damage per wireworm at the high level of infestation is very small. All the evidence thus supports the explanation suggested above.

For those fields on which two plant counts were taken, the interval between the two varied from a week to a month; in spite of the disturbance caused by this variation, it might be

expected that some relationship would exist between the loss in plants during the interval and the degree of wireworm infestation. Almost all fields showed a reduction in plant density, the average losses being 180,000/acre on thirty-two fields in the south, 240,000 on forty-two fields in the west, and 260,000 on forty-three in the north, there being approximately a 20 % loss in all cases. There was a slight indication that the loss was greater in the presence of higher infestations, the regression coefficient being 10.3 ± 5.4 additional plants lost for each additional 1000 wireworms. The smallness of this figure suggests that at the time when the first counts were taken, wireworms had already done their maximum damage in so far as the complete destruction of plants was concerned. Damage caused after this time—and in many cases in 1940 damage continued almost until harvest—might affect the development of the remaining plants but have little influence on their number. This explanation is not fully satisfactory, as it is difficult to understand why such a uniformly high loss of plants should have occurred as a natural competition effect in so short a period, particularly in view of the fact that, as will be shown in § 8, the stand in the south and west was already sufficiently below the optimal as to affect the yield adversely.

TABLE 7. *Plant density and wireworm infestation, south region only*

	No. of fields	Wireworm (1000/acre)	Plants (1000/acre)	Loss in plants per additional wireworm
High infestation	35	1060	760	0.15 ± 0.13
Low infestation	28	272	1120	1.36 ± 0.64

Records of wheat crops were almost entirely confined to the south region, and the only information of value on the wireworm—plant density relationship—is contained in fifteen fields from the South-eastern province. These had an average of 643,000 wireworms/acre and a mean plant density of 530,000/acre. The reduction in stand per additional wireworm was 0.32 ± 0.16 plants; though based on very few fields, the agreement with the average for the south region for oats suggests that the crops may have been about equally susceptible to wireworm attack. The wheat crops recorded were, however, almost entirely winter wheat, whereas the oats were nearly always spring sown.

7. YIELD AND WIREWORM POPULATION

Sampling estimates of yield were also obtained from a number of fields, the technique employed for cereals being the same as that for plant counts, the sample area being cut by hand and afterwards threshed. It was not the intention in this investigation to make any detailed study of sampling variation within the sampling lines, and therefore the samples were generally bulked for each line separately. The sampling error between lines was examined for ninety-eight fields of oats and indicated for the standard error of the mean yield of the two lines a value of about 15 % (or 3 cwt./acre for an average crop), a figure rather higher than had been anticipated.

As with plant density, oats is the only crop for which there are sufficient records for satisfactory conclusions to be drawn. The data were supplemented by yield estimates made visually by the observer or by the farmer, or alternatively from the threshing figures, so that yields for 147 fields in all were obtained. The regression of these yields on wireworm population was found in the same way as for plant density.

The results shown in Table 8 do not lend themselves to so straightforward an explanation as do those of Table 6, probably on account of the less direct influence of wireworm attack

on the ultimate yield. Nevertheless, they indicate that in the region of high infestations average yields were low by comparison with the rest of the country and the effect on yield of changes in the level of infestation was very considerable. The significance of this estimated loss of 1.2 cwt./acre for an additional 100,000 wireworms is undoubted. The apparent *increase* in yield with increasing infestation in the west is within the bounds of the errors of estimation; it seems justifiable to conclude that in neither the west nor the north was there much loss of yield due to wireworm at the low infestation levels normally found there, but there is no reason to suppose that the intensity of wireworm attack was any less than in the south on the few fields which were heavily infested.

Crops which were recorded as complete or partial failures, and were consequently redrilled (with the same or a different crop) or simply abandoned, have been excluded from the above analysis. In the north there were only two such fields of oats, and in the west only one, but failures were comparatively common in the south. The exclusion of all failures in this region will lead to the underestimation of the regression of yield on infestation since the higher

TABLE 8. *Regression of yield on wireworm population*

Region	No. of fields	Wireworm (1000/acre)	Yield (cwt./acre)	Loss in yield per 100,000 wireworm
South	50	574	18.8	1.17 ± 0.36
West	38	397	24.0	-0.40 ± 0.52
North	59	212	22.6	0.45 ± 0.42
All	147	383	21.7	0.38 ± 0.25

TABLE 9. *Oats yield and wireworm infestation; south region only*

No. of fields	Wireworm (1000/acre)		Yield (cwt./acre)
	Range	Mean	
15	— 300	164	23.5
18	301— 600	449	19.0
16	601—1000	806	13.6
15	1001—	1328	8.7
64	All	641	16.7

infestations, at which most of these failures occur, will show too small a proportion of low yields. On the other hand, it is likely that all, or almost all, cases of failure were ascertained, whereas only on approximately half the fields of oats initially recorded were yield figures obtained. Hence to include all failures as having zero yields would be to give them undue weight. Assuming this 50 % ascertainment of yields, unbiased results may be obtained by giving half-weight to all crops recorded as failures. This has been done for Table 9, in which mean yields are given for the south region in four ranges of wireworm population, complete failures being recorded as zero yield and partial failures conventionally as 5 cwt./acre.

Table 9 shows very clearly the steady drop in yield with increasing level of infestation. From the extreme entries, it is seen that the average fall is 1.27 cwt./acre for each additional 1000 wireworms/acre. The removal of the bias from the results of Table 8 has thus slightly increased the estimate of the rate of yield loss.

These considerations of bias do not apply to the analysis of plant density made in § 6 above, since failures in general took place after the date at which the first plant counts were taken and there was therefore no similar bias in the selection of fields for which plant densities were recorded.

By making use of the observer's description of crops for which no numerical estimate of yield was obtained, it was possible to augment the crops available for study by an additional 64. Taking an average crop as 15·8 cwt./acre (Ministry of Agriculture's average for England and Wales, 1929-38), crops heavier than two-thirds average were classified as successful, those below one-third as 'failed', and those intermediate as 'poor'. The assignment to these classes may have been influenced by subjective judgement for borderline cases, but the number of crops for which there was any doubt is too few to have biased Table 10 to any appreciable extent.

Table 10, for which the author is indebted to Mr J. C. F. Fryer, shows in striking fashion the increasing chances of failure as the infestation increases. The entries in Table 10 have been further subdivided by regions, but little additional information is obtained, the west and north being very poorly represented among the populations greater than 300,000/acre and the distribution for the south being of a similar pattern to that for the whole country. However, all the evidence of this and the earlier analyses supports the view that the sowing of oats in the presence of wireworm populations exceeding 600,000/acre is an undesirable risk, and that there is still danger in the range from 300,000 to 600,000/acre.

TABLE 10. *Classification of oats crops and wireworm population*

Wireworm (1000/acre)	Crop result			
	Successful	Poor	Failed	Total
- 300	103	12	3	118
301- 600	31	5	9	45
601-1000	11	7	9	27
1001-	4	6	11	21
All	149	30	32	211

TABLE 11. *Wheat yield and wireworm infestation; south region only*

No. of fields	Wireworm (1000/acre)		Yield (cwt./acre)
	Range	Mean	
8	- 300	146	22·3
11	301- 600	461	17·2
17	601-1000	777	13·3
7	1001-	1816	4·4
43	All	670	15·3

In the south region there were 24 estimations of wheat yields, having a mean of 20·9 cwt./acre, on fields with a mean wireworm population of 494,000/acre. These fields indicated a loss in yield per 100,000/acre increase in infestation of $0·59 \pm 0·39$ cwt./acre. The rejection of crop failures for wheat produces a greater bias than it did for oats, as nineteen fields are in this way rejected. Adopting the same technique as with oats and including them at half weight, Table 11 is obtained. The loss in yield with increasing infestation is shown in this table just as strikingly as for oats, the rate of loss as computed from the extreme entries being 1·07 cwt./acre for an additional 1000 wireworms/acre. The removal of the bias in the estimation of the regression coefficient thus brings the results for wheat surprisingly close to that for oats.

There were altogether fifty crops of wheat which were classifiable in the same way as for oats in Table 10. The 10-year yield average for 1929-38 was 17·8 cwt./acre; and on this

basis Table 12 was obtained. As is to be expected, this table confirms the indications of Table 11. All the available evidence, therefore, supports the view that loss of wheat yield by wireworm damage was very similar in extent to that of oats.

TABLE 12. *Classification of wheat crops and wireworm populations*

Wireworm (1000/acre)	Crop result			
	Successful	Poor	Failed	Total
— 300	10	1	—	11
301– 600	6	3	3	12
601–1000	4	5	8	17
1001–	—	2	8	10
All	20	11	19	50

8. PLANT DENSITY AND YIELD

It might be contended that the relationship between plant density and yield had little direct relevance to the problem under discussion. Whether or not this is the case, an examination of this relationship is of interest as throwing further light on the contrasts between regions discussed in §§ 6 and 7. The analysis was made in the same form as in these sections and Table 13 shows the results by regions for 128 fields.

TABLE 13. *Regression of yield on plant density*

Region	No. of fields	Plants (100,000/acre)	Yield (cwt./acre)	Increase in yield per 100,000 plants	<i>k</i> %
South	45	10.3	19.0	0.83 ± 0.28	45
West	38	12.5	24.0	0.68 ± 0.36	35
North	45	12.3	22.3	-0.25 ± 0.23	-14
All	128	11.7	21.7	0.39 ± 0.17	21

The last column of Table 13 shows the increase in yield per acre corresponding to an additional 100,000 plants/acre expressed as a percentage of the mean yield of 100,000 plants, and is a measure of the extent to which increased tillering and other competition effects fail to compensate for a decrease in plant density. Table 13 confirms the surmise of the last section that changes in stand in the south, where plant densities were low, had considerable influence on the resulting yield, whereas the high plant densities of the north were little affected by similar changes.

It has been found (Finney, 1941) that for sugar beet and mangolds the value of *k* for an average stand is about 50 %. There appears to be little evidence on the corresponding competition effects for cereals, though it might be expected that the tillering propensities of the crop would permit a greater degree of compensation for plant loss. The observations discussed here support this view, as only 55 % compensation was found in the south, where plant densities were low. In the north the high plant densities appear to have permitted complete compensation for loss of stand. Indeed in this region there is a suggestion that the stand actually exceeded the optimal, as a result of the very heavy seeding rates employed, though the use of equally high rates in the west was apparently justified. Possibly equally successful crops would have been obtained in the north had slightly lower seeding rates been used.

The result of subdividing the fields of the south region into high and low infestations is shown in Table 14. Again the fields with less than 500,000 larvae/acre show a behaviour

very similar to that of the fields in the north. The separation of the high infestations shows them to have been of more extreme type than the average for the region, the effect of loss of plants on yield having been for them very serious, as 74 % of the proportionate yield of the plants was lost and only 26 % made up by compensating increased yield of the survivors.

TABLE 14. *Plant density and yield; south region only*

	No. of fields	Plants (100,000/acre)	Yield (cwt./acre)	Increase in yield per 100,000 plants	k %
High infestation	22	8.5	14.1	1.23 ± 0.38	74
Low infestation	23	12.0	23.7	-0.18 ± 0.41	-9

9. DISCUSSION AND SUMMARY

In the first part of this paper a survey is given of the results of sample determinations of wireworm populations on grassland intended for ploughing prior to cropping in 1940. It is shown that there was a very marked decrease in infestation from south to north of the country. The adequacy of the standard sampling technique employed (twenty samples of cylindrical cores 4 in. diameter) in estimating the population of a field is discussed and tables are given to show the margin of error which may be expected when this technique is applied to grassland. The criticism that an average population per field is not sufficient guide to its actual condition is answered by showing that there are few fields in which there is sufficient irregularity in distribution for there to be any question of portions being cropped differently, and that the number of such fields only slightly exceeds its expectation on an assumption of random distribution.

The effect of wireworm infestation on the crop is considered in the second part of the paper. For oats it appears that in the north of the country wireworm populations were sufficiently low and plant densities sufficiently high to permit a high rate per wireworm of damage to stand. On account of the high plant density, however, competition effects were large and decreases in stand may have been largely compensated by increased tillering of the survivors. The net result was that yield showed little dependence on the degree of infestation. In this region heavy infestations were too few for any test to be made of the natural belief that their effect on the crop would be similar to that of similar infestations elsewhere in the country, and hence sharply contrasted with the effects just described.

In the south, on the other hand, the six provinces had very heavy infestation and also low plant densities. Though the poor stand was probably in part due to the high general level of infestation, the use of low seeding rates was probably a contributory cause at the time when the counts were made. By comparison with the north, the rate of plant loss per wireworm was necessarily reduced because of the greater population to be fed. Nevertheless the plant number was too low for adequate compensation for loss to take place. Consequently the repercussions on yield were large, and there was a decrease of 1.3 cwt./acre for each additional 100,000 wireworm/acre. The average yield of the recorded fields in this region was 6 cwt./acre less than in the north. Detailed examination showed that with infestations below 300,000/acre the yield was very similar to that of the north, but was steadily reduced as the infestation increased, the chance of complete failure having been large in the presence of high populations. The yield also showed a considerable dependence on plant density, the average stand having been so low that the compensation for reductions by means of competition effects was small—on the more heavily infested fields it was smaller than is

normally the case with a root crop. Little need be said of the three provinces which have been called the west region except that in general their behaviour was intermediate between the north and the south.

The evidence relating to wheat is scanty and is confined to the south region. The yields, however, agree remarkably closely with the results for oats, there having been a reduction of about 1.1 cwt./acre for an additional 1000 wireworms/acre. The proportion of crop failures was higher than for oats, but the proportion of heavily infested fields was also higher. It seems reasonable to conclude that, in this part of the country, the attacks on the two crops had very similar effects.

For barley the number of fields available in the records is too small for any analysis of the type discussed to be worth while. The data with regard to root crops are equally scanty. The continuation and extension of the survey during 1941 should help to fill some of the deficiencies of knowledge in respect of crops other than oats. The study of the effects of wireworm in the second season after grass may also be begun when the new season's records become available. For the present the deductions from oats are an interesting, though incomplete aspect of the problem of advisory work on wireworm infestation; in applying them it must be borne in mind that they are based on a single, and in many ways exceptional, harvest year, and that the fields recorded may not have been a fully representative selection of the grassland of the country.

The collection of the material on which this paper is based has been carried out by the Provincial Advisory Entomologists and their staffs. To all these the author wishes to express his gratitude, both for the completion of many complicated sheets of records and for assistance during the analysis in the clarification of obscure points. In particular, thanks are due to Mr E. E. Edwards of the University College of South Wales, Mr R. A. Harper Gray of Durham University, Mr W. E. H. Hodson of Reading University, Mr S. G. Jary of the South Eastern Agricultural College, Mr J. R. W. Jenkins of the University College of Wales, Dr H. W. Miles of Manchester University, Mr H. C. F. Newton of the Harper Adams Agricultural College, Mr F. R. Petherbridge of Cambridge University, Mr A. Roebuck of the Midland Agricultural College, Mr L. N. Staniland of the Seale Hayne Agricultural College, Dr I. Thomas of the University College of North Wales, Mr H. W. Thompson of Leeds University, and Dr C. L. Walton of Bristol University. The author is also greatly indebted to Mr J. C. F. Fryer of the Ministry of Agriculture's Plant Pathological Laboratory for continued advice on the biological aspects of the problem studied and, by no means least, to Dr F. Yates of Rothamsted Experimental Station, with whom originated suggestions for most of the statistical treatment of the data.

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ON MEASURING THE EFFICIENCY OF A TRACTOR BY ITS FUEL CONSUMPTION

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TRACTOR-TESTING has so far normally been carried out at tractor-testing laboratories using elaborate and costly equipment which cannot be generally available to most agricultural research stations. Research stations without this specialized equipment may have problems presented to them which involve making reasonably accurate measurements of tractor performance. As an example this Station was asked to investigate how the performance of a tractor carrying out normal agricultural operations depended on the type of driving-wheel used, and in particular to determine the advantages and limitations of pneumatic tyres on a tractor. One of the methods used has given such promising results that it is hoped it will allow other laboratories to undertake research on the field performance of tractors.

The information needed by a practical farmer when discussing tractor performance is first: will the tractor do the work satisfactorily, and if it will can the efficiency of the tractor be increased either by increasing the speed of work or by reducing the fuel consumption or by reducing its rate of depreciation. The investigations made considered only the first two ways of increasing tractor efficiency and perforce had to ignore the third entirely. In particular, it is the object of this paper to describe the methods used for comparing the fuel consumption of the tractor under different conditions of work.

The principle underlying the method employed is already widely adopted in agricultural field trials, namely, to determine not only the treatment mean, the mean fuel consumption in the present case, but also its standard error, so that it is possible to judge if the differences between the mean fuel consumptions obtained when the tractor is working under different conditions can reasonably be ascribed to the different conditions of work or only to mere chance fluctuations.

The first stage in the method consists in measuring the fuel consumption of the tractor over a series of short runs instead of one or two long runs. This should allow an estimate to be made of the variability or the error of the mean, which is unconnected with the factor being investigated. And this condition is vital to the subsequent interpretation of the data. It is, therefore, essential to ensure as far as is possible that the fuel used during all the measurements on one tractor condition is not systematically higher or lower than on another tractor condition for reasons unconnected with that treatment. As an example, suppose the tractor is ploughing and a comparison is being made between the fuel used when the tractor is running with the engine-governor full open and only half open. If all the runs with full governor are made first, and if the soil is gradually becoming heavier to plough for any reason, then it

would appear that the fuel efficiency of the tractor when running with full governor was higher than with half governor, but there would be no way of knowing how far this was due to the ploughing conditions being easier.

The second stage in the method consists in allowing for the effect of any measurable factors affecting the fuel that are unconnected with the factors under investigation. It also often allows one to mitigate the harmful effects of systematic errors of the type mentioned above.

Measurements taken.—In all the experiments described here a Case tractor model C modified CC has been employed, and it has either been ploughing or cultivating. Petrol has been the fuel used throughout. A small auxiliary petrol-tank was fitted to the tractor and the fuel supply went through a two-way tap so that petrol was entering the carburettor either direct from the main petrol-tank when the tap was in one position, or from the auxiliary petrol-tank when it was in the second position. The auxiliary petrol-tank was of brass and was closed on top by a cup which was joined to the tank by a neck about $\frac{1}{4}$ in. in diameter. Petrol was poured in the tank until it came up to the top of the neck. A small brass tube, open to the air, was fitted to the tank just below the neck to allow air to escape without having to blow through the neck. At the side of the tank was an open protected glass tube, of internal diameter $\frac{9}{32}$ in., so that the approximate petrol-level in the tank could be seen.

A run is made in the following way. The engine is warmed up, all paraffin is flushed out of the fuel system and the engine is left running on petrol from the main tank. The auxiliary tank is filled up to the top of the neck. The tractor starts work and when it comes to the beginning of its measured course the two-way tap is turned over so that the engine runs on the auxiliary tank. This tank is then turned off at the end of the course, the tractor is then stopped and petrol from a graduated glass measuring-cylinder is poured into the tank until it comes up to the top of the neck. The amount of petrol so measured is taken to be the amount used during this measured run. It is very important that the tractor should be properly warmed up before the experimental runs start. Not only must the engine be warm but it seems as if the gear-box needs to be used as well. If this precaution is not taken, the first one or two fuel consumptions may be 10–20 c.c. too high, which, on a 200 c.c. run, a fairly typical example, is a serious error.

During a run the following measurements were also taken: the time of the run and the number of revolutions of the land driving-wheel if the tractor were ploughing, or of one of the driving-wheels if it were cultivating. If a third person was present the number of revolutions of the other driving-wheel was also counted. The mean drawbar-pull exerted by the tractor was also always estimated. Two types of dynamometer were tried, both designed and made by the Institute of Agricultural Engineering, Oxford. The first one had a dial on the dynamometer, so that the tractor driver had to keep looking backwards to read it. The second had a pressure-gauge mounted on the tractor in front of the driver. Both types had a device for regulating the damping, and by keeping the gauge reasonably damped it was quite easy to take a reading.

Actually, in all the work the driver's estimate of the mean drawbar-pull was used. The errors introduced by using his estimate will be shown, in the last section of this paper, not to reduce appreciably the accuracy of the final results, and the small loss of accuracy incurred is more than counterbalanced by the great saving of time over measuring up dynamometer charts.

Statistical methods used.—The results obtained by the methods described in this paper are only valid so long as the experiments have valid statistical designs, for these control the validity of the arithmetical calculations needed to extract the required information from the experimental data. The statistical principles that must be observed are described in detail in several publications dealing with agricultural field trials, as well as in Fisher's book on the subject,¹ and, when applied to experiments of the type being considered here, are not onerous. The statistical methods of analysis needed are the analyses of variance and co-variance, and at times multiple regression, and these again are adequately described in many modern publications. For the benefit of non-statistical readers a short description of the purpose of the statistical methods used is given here.

The purpose of the analysis of variance alone is to remove the contributions that the presence or absence of certain definite factors makes to the variations in the quantity being measured, so that the precision of the measured mean is increased. It allows one at the same time to estimate the probability that these factors do in fact influence the variability of the measured quantity. For example, if the fuel consumption of a tractor ploughing up and down a slope is being measured, the application of the analysis of variance allows one to decide if the hill affects the fuel consumption and, if it does, to remove its effect from the variability of the observed fuel consumption, so decreasing the standard error of the observed mean. This removal of the effect of the hill on the variability of the mean fuel consumption does not imply that this mean fuel consumption is the same as would be found if the tractor was ploughing on the level, and this illustrates why the exact significance of the mean must be carefully considered before it is used for further comparisons. In this particular example, as will be shown later, the mean fuel consumption when ploughing up and down a uniform slope does in fact appear to be the same as when ploughing on the level, but this is a fact of experiment and has nothing to do with statistical analysis, though the analysis is essential to increase the precision of the truth of the statement that these two means do not in fact differ significantly.

The analysis of co-variance enables allowance to be made in the quantity under examination for uncontrolled variations in one or more of the associated measurements by obtaining the regression equation connecting them with this quantity, and then adjusting the mean value of this quantity to its expected value had the associated variables been held constant. For example, if the effect on fuel consumption due to varying the type of driving-wheel fitted to a tractor is being studied, the

¹ *The Design of Experiments*, by R. A. Fisher. Oliver and Boyd, Edinburgh.

mean drawbar-pulls in the different experiments will not in general be the same, on account of the heterogeneity of the soil being ploughed. If there is a reasonably close relationship between the fuel consumption and the drawbar-pull, the accuracy of the comparison of the types of wheels will be increased by using this method to adjust the mean fuel consumption for each wheel to the value it would be expected to have if the drawbar-pull had been kept at its mean value throughout the experiment.

There is an important limitation to the class of subsidiary measurements which may be used to increase the precision of the main comparisons in this way. In the example above, the treatment described is appropriate only if the differences in drawbar-pulls for the several types of wheel are the result of mere random variations in the character of the ground being ploughed, and are not themselves caused by the differences in wheels. If an analysis of variance of the drawbar-pulls, on exactly the same plan as for the fuel consumptions—the computation of which is, incidentally, an essential part of the co-variance analysis—shows that there were significant differences in the mean drawbar-pulls corresponding to the different types of wheel, any adjustment of the fuel consumption to a basis of *equal* drawbar-pulls would prove misleading and should not, in general, be attempted. In the particular example given it has in fact been assumed that the draught of the plough is independent of the type of tractor wheel fitted, as it was not possible to test this by controlled experiment. But the only likely cause of dependence is the speed of ploughing, and the draught has been shown to be independent of this within the experimental range. It is highly probable, therefore, that it is legitimate to increase the accuracy of the comparisons of the fuel consumptions by making them at equal drawbar-pull. On the other hand, the time taken to plough a given distance will, in general, be directly affected by the type of wheel used, since different types have different rolling radii and may give different amounts of slip. Hence if the fuel consumptions were adjusted for inequalities in the time of ploughing, one of the most important components of the wheel effect might be removed and possibly a conclusion reached that two tyres were equally efficient when in fact one was better on account of its allowing the tractor to move at a greater speed.

Accuracy of the fuel measurements.—Accuracy of fuel measurement depends upon three separate factors: (i) the accuracy of the actual measurement of the quantity of fuel used, which is solely dependent upon the accuracy of the difference between the two readings of the measuring-cylinder and the accuracy with which the auxiliary petrol-tank can be filled up to a standard condition; (ii) the effect of the variable tilts the tractor may have in the field when the auxiliary fuel-tank is being filled up on the capacity of the tank; and (iii) the constancy of fuel used by the tractor-engine when the tractor is running under constant conditions.

A few experiments have been made on the accuracy with which the tank can be filled from the measuring-cylinders used, which were usually 500 c.c. glass cylinders graduated in 5 c.c. divisions, and on the

influence of tractor tilt. It was found that fuel measurements could be in error by about 1.2 c.c., due to errors of measurement alone, which was increased to about 1.6 c.c. when errors of tilt were superimposed. There has been no opportunity of determining the order of magnitude of the third factor by running the tractor under accurately controlled conditions, as no testing car was available.

Estimates can be made from the results of actual runs of the upper limit of the errors of measurement by calculating the variability of the fuel consumption of the tractor ploughing a measured course when variations in drawbar-pull and time of run are allowed for. It was not necessary for the investigations (for which this technique was developed) to find out by how much this upper limit is in excess of the actual errors of measurement, but it is in excess since there is no reason to suppose that all the effects of the soil and plough variabilities on the fuel consumption have been eliminated by the multiple regression of the speed and drawbar-pull on fuel consumption.

Two examples will be given to show the kind of accuracy of fuel measurement obtained. In the first one the tractor was ploughing a field having a pronounced unsymmetrical ridge running across the direction of ploughing, though the levels at the beginning and end of the run were probably about the same. The tractor pulled a three-furrow plough easily over most of the field in second gear, the mean drawbar-pull was about 1,600 lb., and the mean speed about $3\frac{3}{4}$ miles per hour. The field was not very uniform, the mean pull over the 8-chain¹ course varied from about 1,300 to 1,900 lb., with a large variation of pulls in each run. The mean fuel consumption for the 8-chain run was 256 c.c. In the second example the tractor was again ploughing up and down a slope, but as the mean drawbar-pull was 1,900 lb., the ploughing had to be done in first gear. The ground was very hard wheat-stubble, the soil a stony clay, and the fuel consumption was measured for a $5\frac{1}{2}$ -chain course. The mean speed of ploughing was $2\frac{1}{2}$ miles per hour and the mean fuel consumption 229 c.c. for the $5\frac{1}{2}$ -chain run.

Table 1 gives the results for the various stages of carrying out these calculations. The first row of the table gives simply the standard errors when no corrections whatever are made for the variability of the soil or the field. The second row gives the standard errors of the actual fuel consumption from the values calculated from the regression of time and drawbar-pull, and the third row gives the standard error after allowance has been made for the effect of slope.

TABLE 1. *Estimated Standard Error of Fuel Consumption (in c.c.)*

Soil	Sandy, loose	Stony clay, hard
Tractor gear	Second	First
Uncorrected standard error	21.3	18.4
Corrected for the regression of time and mean drawbar-pull	6.1	8.7
Corrected for slope of ground as well	5.5	5.6

Both these examples show that in practice the fuel is measured with

¹ 1 chain = 22 yards = 20.12 metres.

a standard error of $5\frac{1}{2}$ c.c., or about $2-2\frac{1}{2}$ per cent. Standard errors of lower absolute, though of somewhat larger proportional, amounts are obtained on shorter runs, as is shown in the experiment on which Fig. 1 is based.

Relationship between fuel consumption and drawbar-pull.—An example will serve to show the kind of relationship between the fuel consumption of the tractor and the drawbar-pull. The tractor fitted with 11.25×28 -in. pneumatic tyres was working in first gear and was drawing a culti-

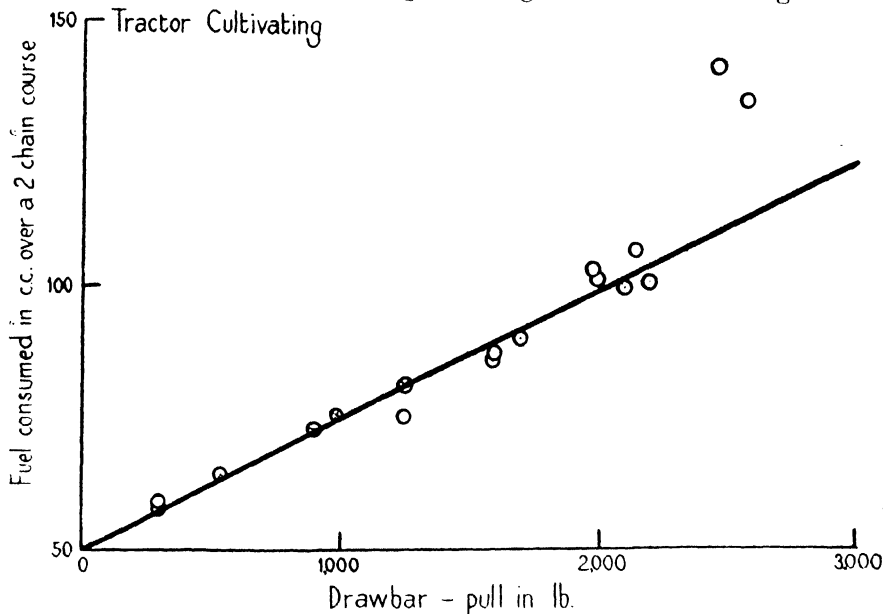


FIG. 1. The relation between fuel consumption and drawbar-pull.

vator through the soil. The fuel consumed to draw the cultivator over a 2-chain course and the mean drawbar-pull were measured, the cultivator was then set either deeper or shallower and a new run was made, and so on. The time was December, the soil was loose and wet, and when the load became too heavy the tractor wheels spun round and the tractor dug itself in. But before this happened the fuel consumption rose abnormally. Fig. 1 shows the result of a typical experiment. The straight line has been fitted to all the experimental points except the last two by the method of least squares. The root mean-square deviation of these points from the line is 3.0 c.c., but the neglected points come 20 and 30 c.c. above the line.

This example shows that the fuel consumption increases linearly with the drawbar-pull from zero pull up to the point at which it increases suddenly either because the engine or the wheels are overloaded, and no exceptions have yet been found for this result. It also shows up the limitation of the method described, for the fuel consumption can be corrected for drawbar-pull only when all such high readings are omitted from the calculations.

This example also gives an answer to the argument that fuel measurements are an inefficient way of estimating the efficiency of the tractor doing a given job because the tractor uses so much fuel when running light that large differences in the efficiency of the tractor when working have only a small effect on the fuel consumption. The argument can be seen to have only a certain measure of validity. Fuel measurements are an inefficient way of comparing the efficiency of a tractor working under different conditions if it is working only at a fraction of its rated horse-power and the wheels are gripping properly. But under these conditions small differences in the tractor efficiency have usually little practical importance. The differences become of practical importance only when they do begin to have an appreciable effect on the fuel consumption or on some other easily measurable factor, such as slip or speed.

Reproducibility of the fuel measurements.—Two sets of data are available to show the kind of reproducibility obtained in these experiments. The first was for a field on a sandy loam at Woburn, Beds., where the fuel consumption was measured when the tractor was working in second gear and drawing a three-furrow plough over an 8-chain course. The moisture-contents of the soil at each date were not recorded, but the firmness of the surface was probably about the same. The fuel consumptions reduced to a constant drawbar-pull of 1,345 lb. were:

<i>Date</i>	<i>Fuel in c.c. per 8 chains</i>	<i>Difference from mean</i>	<i>Standard error of difference</i>
29/5/40	253.3	-2.4	3.4
11/6/40	250.9	0.0	2.5
27/6/40	248.4	2.5	2.7
Mean	250.9

The gradual fall in the mean is probably without significance, for if the first and the last experiments are considered the difference in fuel consumption is 4.9 ± 4.0 c.c.

The second set was made on Broadbalk field at Rothamsted. The soil is a stony clay; the surface was wheat-stubble and was extremely hard and dry on August 28-30, was wet on October 9-10, and was intermediate in the middle of September. The tractor was working in first gear and was drawing a three-furrow plough over 5-chain and $5\frac{1}{2}$ -chain courses. The fuel consumptions per chain, adjusted to a drawbar-pull of 1,910 lb. in c.c. per chain, were:

<i>Date</i>	<i>Fuel per chain in c.c.</i>	<i>Difference from mean</i>	<i>Standard error of difference</i>
28-30/8/40	41.10	-0.73	0.39
11-12/9/40	42.42	0.59	0.50
13-18/9/40	42.05	0.22	0.34
9-10/10/40	41.75	-0.08	0.77
Mean	41.83

The high standard error of the experiments of October 9-10 is due to the fact that only two $5\frac{1}{2}$ -chain and two 5-chain runs were made on that

day, whereas on the others usually 16–20 runs in all were made. If this last run is ignored it is seen that there is a significant difference between the August and September runs of 1.13 ± 0.39 c.c., possibly due to the drier surface in August. This shows the kind of constancy one gets when the determinations are made over an extended period of time.

Sensitivity of the fuel measurements.—Two examples will be given to show that the fuel consumption is sensitive to soil conditions. The first one shows the effect of the slope of the ground. Broadbalk field has a mean slope of 1 in 25 for the bottom $5\frac{1}{2}$ chains and of 1 in 110 for the top 5 chains. All the fuel consumptions have been adjusted to a drawbar-pull of 1,950 lb., the mean for this experiment, because in this experiment there was no connexion between the drawbar-pulls and the directions of ploughing.

	Slope: 1 in 25		Slope: 1 in 110		Difference
	Uphill	Downhill	Uphill	Downhill	
Fuel per chain in c.c. . .	43.99	40.76	43.36	42.60	
Effect of direction . . .	3.23 ± 0.69		0.76 ± 0.70		..
Mean fuel consumption .	42.38		42.98		0.60 ± 0.49

The table incidentally shows that the mean value of the fuel consumed on the up and the down journey is the same on the two ends, so that if an equal number of up and down measurements have been made the mean value for the fuel consumption is practically independent of the slope. This result held true for the other five comparable experiments done in this field.

The second example, also from Broadbalk, shows the effect of the soil surface on the fuel consumption when ploughing. There was a strip of well-worked fallow between two strips of wheat-stubble. The soil surface was naturally much less compact than on the stubble. The following table gives the fuel consumed per chain by the tractor when ploughing up the stubble and the fallow when fitted with four different types of wheel.

Wheel	Mean value of drawbar-pull in lb.	Fuel consumed in c.c. per chain		Difference
		On fallow	On stubble	
A	1,640	45.5	41.1	4.3 ± 0.7
B	1,880	45.7	41.5	4.2 ± 0.6
C	1,700	42.1	38.6	3.5 ± 0.5
D	1,640	45.8	41.5	4.3 ± 1.2

It must be borne in mind that the experimentally measured differences were either smaller than these, or reversed, since the drawbar-pull was higher on the stubble than on the fallow plots. The experiment seems to show that the increase of fuel used on the stubble was not affected appreciably by the type of wheel used.

Occasions can arise, however, when the reproducibility between the runs is poor. For example, during some experiments in which the

efficiency of the tractor fitted with different types of tyre was being examined, the mean drawbar-pull during ploughing began to fall. It was obviously desirable to put up the pull so as to keep the conditions more comparable for the different tyres. But the field had a number of patches of high drawbar-pull which the tractor could only just manage. An attempt was made to keep up the drawbar-pull by continuously adjusting the depth of ploughing, making it shallower on the heavier and deeper on the lighter patches. The adjustment was not very easy to make as the onset of failure of the wheels to grip was very rapid. But the result of the experiment was surprising and definite. There was the same linear relationship between the fuel consumption and the drawbar-pull, but the whole level of fuel consumptions was pushed up by 6 per cent. in one experiment and by 17 per cent. in another.

Optimum length of run.—This is a question of considerable importance, as the amount of useful experimental data that can be obtained from a given area of land will depend on the answer to it. The longer the runs the more accurate would one expect the individual measurements to be, but the smaller will be the number of runs that can be made in a given area. Since the amount of land available for this kind of work is usually limited, it is very important to utilize it with the highest efficiency. Unfortunately this point could not be fully explored when this work was in progress, since owing to special circumstances it was almost irrelevant for the greater part of the investigation being undertaken, so that no extensive data were obtained which would help to answer this question.

Only one direct comparison of three different ploughing lengths, of 2, 3, and 5 chains respectively was made, and that was when the tractor was drawing a three-furrow plough and ploughing up an old pasture field. If any correlation between the fuel consumed and the mean drawbar-pull is ignored, there are no differences between the standard errors over the three distances, but if the correlation is allowed for, the standard error of the 5-chain run becomes lower than those of the other two, because it is only on this run that there is any appreciable correlation. The results were:

<i>Length of run</i>	<i>Mean D.-B.P. in lb.</i>	<i>Mean fuel in c.c./chain</i>	<i>Standard error of fuel per run in per cent.</i>		<i>No. of runs made</i>
			<i>Allowing for D.-B.P.</i>	<i>Ignoring D.-B.P.</i>	
2 chains . . .	1,310	35.1	3.6	3.7	20
3 chains . . .	1,380	36.3	3.6	3.8	36
5 chains . . .	1,440	35.7	2.7	3.3	24

This apparent effect of the presence of correlation between the drawbar-pull and the fuel consumption for the 5-chain runs and the lack of it for the 3- and 2-chain runs is probably due to the fact that the variations of drawbar-pull within the runs were small, the root mean-square deviation of the drawbar-pull from its mean being between 55 and 60 lb. on mean pulls of 1,300–1,500 lb. Under these conditions the small effect

of the variability of drawbar-pull on fuel consumption can be shown up only on the longer runs. If the variations of the drawbar-pull are larger than this, as they have been on nearly all the other experiments done here, then the correlation between drawbar-pull and fuel consumption can be shown up on runs of 2 and 3 chains. Fig. 1 serves as an illustration of this, as the data on which it is based were obtained on runs of only 2 chains long.

Hence, although no definite conclusions can be drawn from this experiment, it does appear that there is no considerable advantage in having runs longer than 2 to 3 chains. All the other experimental evidence available supports this conclusion, as the percentage standard errors of the fuel consumption per chain for different fields, using lengths of run from 2 to 8 chains, do not seem to depend on the length of run employed.

Two Examples of the Method

(1) *Effect of gear ratio on fuel consumption.*—Two experiments were made to compare the fuel consumption of the tractor per chain when ploughing in first and second gear. The first one was done on arable land that had carried a crop of kale which had failed, whilst the second was done on a pasture.

TABLE 2. *Effect of Tractor Gear Ratio on Fuel Consumed per Chain when Ploughing*

(a) *Arable land.* Tyres used 11.25×28 in. Recommended deflections.

Load on land wheel (in cwt.)	12½	14½	16½	
<i>Fuel consumption in c.c. per chain adjusted to a mean drawbar-pull of 1,960 lb.</i>				
Tractor in first gear	44.3	43.0	46.6	..
Tractor in second gear	40.7	39.8	40.7	Mean
Difference	3.6	3.2	5.9	4.8 ± 0.6

(b) *Pasture land.* Tyres used 11.25×28 in. Deflected 50 per cent. less than recommended.

Load on land wheel (in cwt.)	12½	16½	$12\frac{1}{2} - 16\frac{1}{2}$..
<i>Fuel consumption in c.c. per chain adjusted to a mean drawbar-pull of 1,600 lb.</i>				
Tractor in first gear	43.7	40.4	3.3 ± 1.1	..
Tractor in second gear	35.5	36.3	-0.8 ± 0.8	..
Difference	8.2 ± 1.1	4.1 ± 0.6

There is clearly a considerable saving in fuel when working in second gear, and this is established quite definitely. There also appears to be a small effect of ballast on the fuel consumption when working in first gear, which is barely significant. The reason it goes different ways in the two experiments is presumably due to the $16\frac{1}{2}$ cwt. load being too heavy for the soft arable and the $12\frac{1}{2}$ cwt. load rather too light for the firm and rather slippery pasture.

(2) *Effect of speed of ploughing on fuel consumption.*—The Case tractor used was fitted with a governor, and experiments were made to see how far the speed of ploughing affected the fuel consumption. The primary reason for doing this experiment is that if one is studying how the fuel consumption of the tractor when ploughing depends upon the type of driving-wheel used, the tractor will normally be ploughing faster the larger the wheel used. Ideally, one should alter the gear ratio in the tractor gear-box in such an experiment so that the forward speed of the tractor is the same for the different wheels. But in the experiments actually made here this could not be done, so that the comparison of different wheels necessitated the tractor working at different speeds.

The speed of ploughing was altered by varying the setting of the governor on the tractor, and in the experiments described here only the so-called full-open and half-open settings were used. Five adequate sets of comparisons were made in all and the results are given in Table 3. The first four comparisons were made on a sandy soil at Woburn, with the tractor working in second gear, and the fifth on a stony clay at Rothamsted with the tractor working in first gear, but in all five the soil was fairly loose before ploughing. In the table the fuel is given in c.c. per chain and the speed in miles per hour.

The mean observed fuel consumptions have been adjusted to allow for the small differences in mean drawbar-pulls for the full- and the half-open governor settings. This is legitimate because the drawbar-pulls are independent of the governor setting, and therefore of the speed of ploughing, as is shown in the second row of the table. The last row of the table shows the loss of precision that would have occurred if the drawbar-pull was not independent of speed, so that the effect of drawbar-pull could not have been allowed for.

These results show quite clearly that variations in the speed of ploughing have no appreciable effect either on the draught of the plough or on the fuel consumption of the tractor per acre. They show clearly the reason why the most economical method is to work the plough at the greatest speed at which it can do passable work.

Accuracy of estimation of the drawbar-pull.—In all the experiments described here the drawbar-pull was estimated by the tractor driver. He usually had an oil pressure-gauge in front of him, and after ploughing or cultivating his measured course he gave his estimate of the drawbar-pull exerted by the tractor during that run. He usually gave his estimate in units of 50 lb., though on a few occasions when the pulls were very even he gave them to 25 lb. This procedure was only possible by smoothing out the very short-period oscillations of the dynamometer needle. The damping adjustment is fairly critical, for if the gauge is damped down too much very misleading readings can be given. In use the damping was adjusted so that the pointer took between 2-3 seconds to reach its maximum value from zero.

In the earlier experiments, as already stated, the dynamometer-dial was attached to the connecting link itself and the tractor driver had to turn round to read it. On several separate occasions, while the tractor was ploughing an 8-chain course, another observer walked beside the

TABLE 3. *Effect of Speed on the Fuel consumed when Ploughing*

	A		B		C		D		B	
	Fuel c.c./chain	Speed m.p.h.	Fuel c.c./chain	Speed m.p.h.	Fuel c.c./chain	Speed m.p.h.	Fuel c.c./chain	Speed m.p.h.	Fuel c.c./chain	Speed m.p.h.
Wheel used										
Place of experiment										
Mean drawbar-pull in lb. = P										
P (full governor) — P (half-governor).										
Governor full open	34.29	3.1	30.98	3.4	29.98	3.7	29.46	3.7	41.51	2.5
Governor half open	34.16	2.4	30.73	2.6	29.74	2.8	28.87	2.8	40.98	2.0
Full minus half-governor:										
Corrected for P	0.12 ± 0.70		0.25 ± 0.34		0.24 ± 0.61		0.59 ± 0.29		0.53 ± 0.46	
Not corrected for P	-0.58 ± 1.60		1.03 ± 0.61		-0.01 ± 1.06		0.30 ± 0.65		0.90 ± 0.70	

link and entered the dial-reading every 6 to 10 yards. The mean value of these 17-24 readings was taken as the mean drawbar-pull over this run. The tractor driver was found to over-estimate the pull by about 20-25 lb. over a series of 50 comparisons on this basis. In one series his standard deviation over this mean was about 45 lb. and in the second about 30 lb., the difference between the two series being due to the more even pulls in the second set. In the first set the average maximum variation of pull in a row was about 600 lb., and the maximum variation in the mean pull from row to row about 900 lb. In the second set the two figures were 450 lb. and 600 lb. respectively, so the whole range of variabilities was smaller in this set than in the first.

These errors in estimating the drawbar-pull naturally increase somewhat the apparent error of the fuel measurements. But the effect is not very large. In the first set of experiments, when the standard deviation in the estimate of error was 45 lb., the apparent standard error in the fuel consumption per run was about 11.0 c.c. It would have been reduced to 10.0 c.c. if the drawbar-pull had been estimated correctly. In the second set, in one series the standard error of the fuel consumption per run would have been reduced from 11.3 to 11.0 c.c. for one set of determinations and from 15.5 to 14.9 c.c. in another.

It appears therefore that the tractor driver was able to estimate drawbar-pulls over runs of as long as 8 chains with a standard error of between 30 to 50 lb., depending on the variability of the soil, and that this error causes only a negligible increase in the residual errors of the fuel consumption.

Summary

An investigation has been made on how accurately the fuel consumed by a tractor running over distances of 2 to 8 chains (44 to 176 yards) can be measured. It has been shown how the crude measurements can be corrected for certain variable factors, and that when this has been done it is comparatively easy to measure the fuel consumed to about 3-5 per cent. per run.

Examples are given of the kind of results that can be obtained and the precautions that must be taken to ensure that the results are correctly interpreted.

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CROP RESPONSE TO INTER-ROW TILLAGE¹

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Is the purpose of intertillage of wide-spaced crops solely to control weeds or has the mulch, which is produced as a by-product of the weed-destruction, any beneficial effect on the crop? It is generally agreed that the fundamental purpose of intertillage is weed-control, but there is a firm belief in practical farming circles that the mulch produced by the hoeings is of itself of great benefit. The purpose of this paper is to examine the available data to decide if mulching, *per se*, has this supposed beneficial effect on the crop, for if it has not, then it will be worth while investigating if the mulch-producing methods of hoeing and grubbing are the most economical or efficient way of weed-control. In particular, a scraper or soil-sweep that only disturbs the top inch of soil without producing any appreciable surface mulch might be a more efficient and economical way of weed-control than the deeper cultivation that is done by hoes or grubbers. Agricultural experiment stations in the U.S.A. have published, during the last half-century, a formidable volume of literature on the subject. Much of the early work included little or no replication, but the contrast between soil scraped to destroy weeds and soil stirred with implements has been a common factor in a large number of the experiments. These have included widely differing conditions of soil and climate. This range of conditions is highly desirable in testing any question in the field, and valuable information may be obtained from a survey of the published results of this series of simple plot trials.

1. *Experiments with Maize in the U.S.A.*

This evidence has for long been considered inapplicable to British agriculture, since most of the trials were conducted with maize (field corn), a crop grown in rows about 3-4 ft. apart and of no economic importance in Britain. Also the Great Plains, where much of the early work was done, have extremes of climate which are rare in British experience. It is, however, under occasional drought conditions in Britain that 'dry farming', i.e. inter-row tillage aimed at moisture-conservation, is most strongly advocated. This evidence cannot be ignored entirely. Further, as the tillage methods for maize are used for other crops too, the evidence cannot be completely disregarded, and is therefore surveyed here.

Only those experiments of the most adequate duration, and in some cases replication, are included in Table 1. (The letters in the right-hand column refer to the subsequent commentary.)

Notes on Table 1.—(a) This is probably the first record by an experiment station of a field study of the inter-row tillage of crops. Root-

¹ Abridged from a Thesis approved for the Ph.D. degree by the University of London.

studies were attempted and much root-damage by cultivation implements was reported. Sturtevant,¹ in setting out his results, commented: 'Strangely enough, we have . . . been unable to obtain decisive evidence in favour of cultivation.'

(b), (c), and (d) This unexpected failure of inter-row tillage to produce effective increases in crop yield was observed at other experiment stations, over a course of several years of simple plot trials.

TABLE I. *The Effect of Intertillage compared with Weeding on the Yield of Maize*

<i>Experiments with field corn</i>			<i>Results: Grain Yield in Bu./Acre</i>				
<i>Year of publication</i>	<i>Experiment Station</i>	<i>Duration in years</i>	(i) <i>Weeded no inter-tillage</i>	(ii) <i>Normal inter-tillage</i>	(iii) <i>Extra deep or frequent inter-tillage</i>	<i>Per cent. increase for (ii) over (i)</i>	
1887	Geneva, N.Y. ¹	4	70·5	56·8	..	-36·0	(a)
1897	Illinois Unis. ²	7	69·1	70·79	..	+ 2·4	(b)
1900	Utah ³	5	64·03	60·15	62·12	- 6·0	(c)
1900	South Carolina ⁴	2	62·00	62·95	..	+ 1·5	(d)
1912	28 States ⁵	7	99·1%	100%	..	- 0·9	(e)
1915	Minnesota ⁶	3	64·17	49·15	62·71	-23·4	(f)
1915	Illinois ⁷	8	45·9	39·2	..	-14·6	(g)
1917	Virginia ⁸	4	49·0	59·4	58·6	+21·3	(h)
1918	Kansas ⁹	4	38·6	40·4	38·2	+ 4·7	(i)
1925	Illinois ¹⁰	6	54·5	55·6	55·3	+ 2·1	(j)
1925	Arkansas ¹¹	19	36·3	38·6	36·7	+ 6·3	(k)

Mean percentage increase for cultivations—3·8.

(e) As a result of the above evidence the U.S. Department of Agriculture organized a standardized trial, over a wide range of both soil and climatic conditions. On two 80-yard 5-row plots the mere removing of weeds by scraping was compared with the supposedly ideal cultivations. No weeds were allowed to grow on either plot. The results were summarized by Cates and Cox⁵ in 1912. Although the scraping treatment produced some apparently unfavourable conditions, such as soil surfaces 'dry and hard as a floor', there was a difference of less than 1 per

¹ Geneva (N.Y.) Agric. Expt. Stat., *Ann. Rept.* 5, 1886, 46.

² Ill. Univ. Agric. Expt. Stat., 1892-6, *Bull.* Nos. 20, 25, 31, 46.

³ Utah Agric. Expt. Stat., 1900, *Bull.* No. 66.

⁴ S. Carolina Agric. Expt. Stat., 1900, *Bull.* No. 61.

⁵ U.S. Dept. Agric. Bur. Plant Ind., 1912, *Bull.* No. 257.

⁶ Univ. Minn. Agric. Expt. Stat., 1915, *Bull.* No. 149, Part I.

⁷ Ill. Agric. Expt. Stat., 1915, *Bull.* No. 181.

⁸ Virginia Agric. Expt. Stat., 1917, *Bull.* No. 214.

⁹ *J. Amer. Soc. Agron.*, 1917, 9, 49.

¹⁰ Ill. Univ. Agric. Expt. Stat., 1925, *Bull.* No. 259.

¹¹ Univ. Ark. Agric. Expt. Stat., 1927, *Bull.* No. 219.

cent. in the grain-yield means for the 112 experiments. These had been conducted in 28 States, in widely varying soils and climates:

Year	Grain		Fodder	
	Number of experiments	Average yield of scraped, as percentage of cultivated plots	Number of experiments	Average yield of scraped, as percentage of cultivated plots
1905+6	5	108.4	3	96.36
1907	8	110.84	2	91.6
1908	6	97.93	4	94.95
1909	25	105.27	10	101.15
1910	43	96.51	19	96.19
1911	25	92.37	15	91.24
Weighted means	(112 expts.)	99.108	(53 expts.)	95.1

The importance of these experiments lies in the wide range of soil types involved. These varied from 'fine sandy soil' to 'stiff yellow clay', and the sites ranged from the coast to the central plains. As the data gave a distribution-curve of normal type, Cates and Cox inferred that the results were probably reliable. Rainfall had little effect. The soil mulch appeared to be equally unremunerative on fertile and on infertile soils, for the ratio of unmulched to mulched crops was 97.19 per cent. for all grain yields below 30 bushels, and 98.34 per cent. for all yields above 50 bushels per acre. The results were interpreted as showing no important effect from soil mulching beyond that of weed-destruction. Mosier and Gustafson,¹ studying these results, raised a point of great interest three years later by showing that the mulch appeared to affect the yield favourably on heavy soils, but unfavourably on light soils.

(f) and (h) represent the results of more detailed studies in continuation of those summarized in (e). They show approximately equal and opposite effects of normal inter-row tillage, but both show negative results for more deep or frequent soil stirring.

(g) Mosier and Gustafson¹ included a study of weed-competition. They irrigated some weedy plots, but this only partially remedied the weed-damage, showing that factors other than soil moisture were important in weed-competition.

(i) Call and Sewell² concluded that weed-destruction was the major purpose of inter-row cultivation.

(j) Wimer and Harland³ continued for six years the work of Mosier and Gustafson, and compared both medium and shallow inter-row tillage with scraping. They concluded that on their silt-loam soil such tillage should be shallow, and given only for weed-destruction.

¹ Ill. Agric. Expt. Stat., 1915, *Bull.* No. 181.

² *J. Amer. Soc. Agron.*, 1917, 9, 49.

³ Ill. Univ. Agric. Expt. Stat., 1925, *Bull.* No. 259.

(k) Nelson and McClelland¹ summarized in 1927 the results of nineteen years' continuous trials of a simple pattern in which only the controls were replicated. They reported an average loss of 81 per cent. of the crop when weeds were not destroyed, whilst the five different cultivation treatments produced average variations of less than 6.5 per cent. Experiments on heavier soils by Sachs² were inconclusive, duplicated control plots varying by over 100 per cent. in each of the four years.

The experimental data, summarized above, illustrate a great many cases where inter-row tillage beyond that essential for weed-destruction has given no remunerative return, which is particularly emphasized by column (iii) in Table 1. These experiments justify the conclusion that, for the maize crop, inter-row cultivations beyond those necessary to keep the crop clean are unlikely to produce positive economic returns except in special circumstances. This failure of an important American crop to respond to the traditional methods of inter-row tillage is a challenge to the hypotheses on which this tillage is advocated.

2. *Experiments with Vegetable Crops in the U.S.A.*

Thompson,³ of Cornell University, N.Y., published in 1927 the results of six years of well-replicated plot experiments on the inter-row tillage of vegetables growing in a sandy loam soil. Surface-scraping was compared with the maintenance of a mulch by weekly hand-stirring of the soil. He studied and photographed the root-systems of six vegetables, in search of a relation between the character of the root-system and the response of the crop to mulching cultivations.

Carrots, cabbages, and tomatoes showed no significant increases in yield when mulched, but significant increases were obtained for beet (4.25 per cent.), onions (7.6 per cent.), and celery (24.1 per cent.). Only with celery were the increases consistent. With both the beet and onions larger yields were produced in some years on the scraped plots. Weed-competition on some extra plots caused heavy losses. Thompson concluded: 'The yield-data show conclusively that weed-control was of much greater importance than the maintenance of a soil mulch.'

His root-studies on beet revealed a lateral root-spread of 3-4 in. when the plants were 6 in. high. At a height of 12 in., when the diameter of the young beet was about 1 in., the soil between the rows was filled with fine roots to a depth of 4 in. Throughout the growth of the plants, root-development was at a maximum in the first 3 or 4 in. of the soil. Thompson concluded that for beet, 'cultivation with ordinary implements would cause considerable injury at any time after the first few weeks'. The root-systems of the vegetables partly explained their response to inter-row tillage. His results for beet are given above in some detail, because vegetables of a similar type form a large part of the British 'root-break'. Cultivation of such vegetables is evidently liable to produce considerable root-damage in the middle part of the growing-season. This damage is not visible from the surface, and is not easily detected.

¹ Ark. Univ. Agric. Expt. Stat., 1927, *Bull.* No. 219.

² Ark. Univ. Agric. Expt. Stat., 1926, *Bull.* No. 205.

³ Cornell Univ. Agric. Expt. Stat., 1927, *Mem.* No. 107.

In 1923 the experiments were extended to a contrasting soil type. Thompson, Wessels, and Mills¹ published in 1931 the results of the second series of experiments on a silt loam on Long Island (N.Y.). Their results for the potato crop are discussed in section 4 below. Five years of experiments with carrots, four with cabbages, onions, and potatoes, and seven with beet and tomatoes, all on this heavy soil, led them to the conclusion that 'the main advantage derived from inter-row cultivation was due to weed-control, and that the formation and maintenance of a soil mulch did not significantly increase the yield of any one of the six crops'.

In 1938 leading members² of the U.S. Department of Agriculture summed up the experience of the Department in the following generalizations: 'Tillage of growing crops is now regarded primarily as a means of weed-control, as it is becoming better recognized that the chief purpose of cultivation is to destroy weeds, not to create a mulch.' With specific reference to the potato crop they consider this to need 'some ridging to protect the tubers, although, in general, level cultivation is preferable to ridging which involves deep tillage and root pruning. The labour of inter-tillage, and the total tillage costs, may be reduced by cultivations before the crop is planted.'

3. *Experiments with Roots and Kale in Great Britain*

As yet very little experimental work on inter-row tillage has been carried out in Britain. Interesting results, which were not published, were obtained in 1907 and 1908 at Reading, when the effects of weed-competition and hand-hoeing were studied for the mangold crop. After applying 10 tons per acre of dung and 7 cwt. per acre of fertilizer in each year, the results from an area of half an acre were:

<i>Treatment</i>	<i>Yield in tons per acre</i>	
	1907	1908
9 rows. No weeding after setting out of plants	15½	16½
10 rows. Hoed once only	33½	30½
10 rows. Hoed twice only	37½	36½
10 rows. Kept completely clean by several hoeings	39½	38
9 rows. Kept clean by hand-weeding only, no hoeing	40	38½

A single hoeing appeared nearly to double the crop, a second hoeing to add another 5 or 6 tons, whilst frequent hoeings appeared to produce little more than 1 ton further increase. The rows kept clean by hand-weeding surpassed the remaining rows in both years, suggesting strongly that the benefit of the hoeing lay in the killing of weeds rather than in the improvement of soil texture. The consistency of the results for the two years indicates that they are probably reliable, although the lack of statistical design renders impossible any accurate estimate of experimental error.

The first British field studies of inter-row cultivation to be conducted

¹ Cornell Univ. Agric. Expt. Stat., 1931, *Bull.* No. 521.

² U.S. Dept. Agric., *Yearbook*, 1938, p. 321.

with statistically controlled designs were laid down at Rothamsted Experimental Station in 1932 and have been described by Keen and Russell.¹ Trials were carried out with kale and sugar-beet on the heavy Rothamsted soil, and with sugar-beet on the sandy soil of the Woburn Experimental Farm. No economic advantage was obtained from intensive hoeing in any one of the five experiments, and in three of the five this extra work both increased growing-costs and reduced yields. These English results are in accord with the American ones, but they contrast sharply with the widely held traditional views on the value of stirring the soil while such crops are growing. Naturally, a large series of experiments will be needed before the influence of seasonal and other factors can be fully defined, and occasional conflicting results may be expected, especially on light land.

4. *Inter-Row Tillage for the Potato Crop*

In their large amount of field work on tillage the numerous agricultural experiment stations seem to have overlooked the potato crop until quite recently, although Sturtevant² in 1882, working at Geneva, N.Y., showed that ridging gave no advantage over flat culture on a 'rather wet clay soil'. He further studied the root-system of potato plants grown under different systems of cultivation and concluded that 'for the potato plant a system of cultivation which interferes with the root-system is a disadvantage'. The U.S. Department of Agriculture in 1921 wrote: 'The objects of tillage are to prevent weed-growth, conserve moisture, aerate the soil, increase the available supply of plant-food, and stimulate root-growth.'³

The experiments of Thompson, Wessels, and Mills⁴ on inter-row tillage covered the four years 1927-30; they were done in Long Island, N.Y., on a well-manured silt loam, with gravel at 3-4 ft. The three tillage treatments were: (1) scraping with a sharp hoe; (2) shallow cultivation with a 'garden tractor' 4 to 6 times; (3) shallow cultivation 8 to 9 times. There were three replications and all weeds were eliminated from all plots. The potatoes were not earthed up. The mean results for the four years were:

Cultivation treatment . . .	(1)	(2)	(3)	Weedy plot
Total yields in lb. per plot . . .	158.82	150.42	148.35	52.67

The yields of the scraped plots (1) were significantly greater than those of (2) or (3), and this effect was consistent, e.g., of the twelve comparisons provided by three replications in four years, eleven favoured the scraping treatment. The rainfall in the district varied from 26.7 to 30 in. in the four years.

They concluded 'that maintenance of a soil mulch by cultivation was

¹ *J. Roy. Agric. Soc.*, 1937, **98**, 53.

² Geneva (N.Y.) Agric. Expt. Stat., *Ann. Rept.*, 1882.

³ U.S. Dept. Agric., Bur. Plant Ind., 1921, *Farmers' Bull.* No. 1190.

⁴ Cornell Univ. Agric. Expt. Stat., 1931, *Bull.* No. 521.

of no value, or else that the cultivation was injurious, and offset any advantage that might have been derived from the mulch'. They reported much greening of tubers, and therefore suggested that ridging was necessary, but it should be moderate, so that cultivation could be kept as shallow as possible. The soil type and the total precipitation in these experiments are similar to those occurring in parts of Britain, and the results should, therefore, be applicable to some British conditions.

Merkle and Irvin,¹ in 1931, published results of five years' work in Pennsylvania on the inter-row tillage of various vegetable crops, including potatoes. They worked on a heavy silt loam, well drained, with no water-table within measurable depth. Their three cultivation treatments were: (1) scraping with a sharp hoe, (2) three cultivations 2-3 in. deep, (3) six to eight similar cultivations. The treatments were replicated 2, 6, 4, 4, and 3 times respectively in the five years of the experiment. Only in the one exceptionally dry season of 1930 (half the normal rainfall) did the cultivation treatments produce any significant result. Treatment 3 then showed some advantage both in yield and in moisture-content. Merkle and Irvin concluded: 'The results reported here do not discourage any practice which will keep weeds under control. They do show, however, that when weeds are kept under control further cultivation of potatoes for other purposes is not essential.'

The publication of Thompson's results in 1927,² already described, led to the conducting of field trials in Maine (one of the leading potato-producing States of the U.S.A.) by the U.S. Department of Agriculture, in 1931-3. Lombard³ reported the results in 1936. Four replications were used. Three-year-means for yield are given below.

<i>Treatment</i>	<i>Total yield bu./acre</i>	<i>Percentage of ware</i>
1. Harrowing (2 weeks after planting), one grubbing and one early high ridging	365.6	92.4
2. Harrowing, five grubblings, moderate ridging	360.3	92.2
3. No harrowing, two grubblings, moderate ridging	364.0	92.0
4. No harrowing, three grubblings, moderate ridging	351.0	91.7
5. No harrowing, four grubblings, moderate ridging	360.8	93.0

All weeds were removed by the hand-hoe. None of the differences was statistically significant, and the comparison of treatments 1 and 2 shows that the four additional grubblings received by 2 did not improve the yield. The soil type is not mentioned in the report. Lombard concluded that weed-control was the only important effect of inter-row cultivation.

Results of a very extensive study of tillage methods for potatoes were published by Moore,⁴ of Cornell University, in 1937. He reported trials

¹ Penn. Agric. Expt. Stat., 1931, *Bull.* No. 272.

² Cornell Univ. Agric. Expt. Stat., 1927, *Mem.* No. 107.

³ *Amer. Potato J.*, 1936, **13**, no. 9.

⁴ Cornell Univ. Agric. Expt. Stat., 1937, *Bull.* No. 662.

of (1) level culture, (2) 4-in. ridging, (3) 7-in. ridging, all duplicated for the comparison of (a) scraping with a sharp hoe, (b) cultivation with a one-horse grubber, working at a depth of 3-4 in. every 10 days until the blossoming of the plants. The experiment included the three growing-seasons of 1932-3-4. Four replications of 30-yard three-row plots were employed, the row distance being 36 in. The soil is described as a well-drained very heavy silt loam.

Elaborate studies of the root-development were made at all stages of the plant-growth, and the effect on the roots of inter-row grubbing was clearly shown. Moore found that 'at the age when the tops are only 6 in. to 8 in. tall, the lateral spread is sufficient to be subjected to severe pruning by cultivation'. He investigated the possibility that the pruning of lateral roots by tillage might result in the deeper penetration of the remaining roots. He found that when the roots 4 in. from the row were pruned to a depth of 4 in., the remaining roots reached a depth of 29 in., whilst the roots of unpruned plants reached 33 in. He observed that the potato roots recovered fairly rapidly after such pruning, to re-establish themselves in the rich surface soil. Thus 24 days after cultivation had ceased he found that 'mid-way between the 36-in. rows the surface soil for the first 6 in. was filled with fibrous roots, the upper half of which were branches of roots pruned by cultivation'. (Roots in the scraped plots filled the entire surface 6 in. of soil, whether level or ridged.) The only difference then visible between roots on scraped and grubbed plots was that 'under cultivation the roots between the rows in the upper 3 in. of soil were almost entirely branches arising from the pruned roots'.

The yields were greatest with level culture, decreasing with increasing height of ridge. This Moore attributed mainly to the decrease in the moisture-content of the first 12 in. of soil with increasing height of ridge. A very high correlation was observed between yield and moisture-content. Grubbing appeared to have no effect on yield when the potatoes were grown in ridges, but there was a suggestion that it reduced the yield on the level culture. He found that protection of the tubers from greening was satisfactory when broad ridges only 2 in. high were made. Moore concludes that 'deep cultivation at any time, except possibly before the plants are 2-4 in. high, cannot be justified from the evidence here presented. Any stirring of the soil that exceeds 1 in. in depth is accompanied generally by a certain amount of root-pruning.'

Lähde,¹ of Finland, in 1938, described some experiments comparing ridging with flat culture, and showed that:

(i) Whenever the grubber or horse-hoe is used within 30-35 cm. of the plants in the later stages of plant-growth, some root-damage is done, which increases as the implement works nearer to the plants.

(ii) The lateral spread of the tuber-bearing stolons was found to vary greatly in different Scandinavian varieties of potatoes. He gives photographs of the washed root, tuber, and stolon systems. In the varieties with the greater lateral spreading of stolons, the use of inter-row tillage implements in the later stages of plant-growth causes damage to the stolons resulting in reduced yields. A 7-year series of trials at the Agri-

¹ *Valt. Maatalousk. Julk.* (Helsinki), 1938, no. 98.

cultural Experiment Station at Tikkurila (Finland), on sandy soil, showed mean yield increases of 7.5-9 per cent. over the uncultivated controls, by a single early earthing-up. This increase was reduced by a second earthing-up, and completely negated by a third such operation. Deep inter-row tillage, in the early stages of plant-growth, with a 'Pelturi' grubber, gave slight decreases in yield on sandy soil, but slight increases on clay soil. No significant effects of tillage were detected in the starch-content of the tubers. Lähde also quotes experiments at the Piikkio Station in 1931 and 1932, on a sandy soil, comparing flat culture with ridging, and other trials in 1934-6, on both clay and sandy soils at Nordhame, comparing ridging, superficial hoeing, and deep grubbing. The results of both series showed no differences greater than the experimental error. A single year's experiment at Süd-Pohjanmaa, on a clay soil in 1933, gave results distinctly favouring earthing-up once, at an early stage of plant-growth, as compared with horse-hoeing four times, earthing-up four times, or a combined treatment. Several more examples, giving similar results, are described.

Lähde concludes that ridging and level culture have given, on the average, equal yields. He prefers, however, ridged to level culture for practical reasons, since it reduces greening of tubers and, in his opinion, facilitates weed-destruction and harvesting. The ridging must, however, be done early, when the plants are 10-20 in. high, and only one such ridging should be given. If weed-growth makes a second ridging necessary, this must be given before the plants bloom.

Apart from scientifically planned trials, useful evidence may be gathered from the records of Growers' Clubs, membership of which is restricted to those growers whose crop yields attain a definite minimum standard. Dickey, in 1935,¹ gave an account of the '400 Bushel Club' of Pennsylvania, which had then been meeting for seven years. He showed that in spite of environmental difficulties the methods of the most successful growers had tended towards standardization: 'The average number of inter-row cultivations has decreased from 3-5 in 1923 to 2-3 in recent years. Growers are coming to a better realization of the theory of cultivation, and its possibilities and dangers, and are making much greater use of the harrow before emergence of the crop, and of the weeder (an instrument bearing light spring-tines of a type similar to those of a hay-rake, several of which operate between each row). The percentage using a weeder has risen to approximately 90.' This type of evidence has a ready appeal to the practical farmer, and similar organizations for potato growers elsewhere would serve a very valuable purpose.

The first data on inter-row tillage of potatoes to be obtained in England were published in the Rothamsted Report for 1932, when Mr. H. Stewart gave the results of a randomized-block experiment carried out at Kingennie, Angus. On a medium loam four randomized blocks, of eight plots each, were laid down to include four levels of application of ammonium sulphate, with two levels of intensity of cultivation. A heavy crop was obtained (an average of 14.13 tons per acre of the Kerr's Pink variety), but no improvement in yield resulted from the additional inter-

¹ *Amer. Potato J.*, 1935, no. 12.

row working. Expressed as percentages of the general mean, the treatment yields were:

<i>Ammonium sulphate cwt./acre</i>	<i>0</i>	<i>1</i>	<i>2</i>	<i>3</i>	<i>Mean</i>
Normal cultivation . . .	98.6	100.1	100.3	102.9	100.5
Intensive cultivation . . .	93.6	97.4	103.4	103.6	99.5
Intensive—Normal . . .	—5.0	—2.7	3.1	0.7	—1.0

Thus the additional cultivations, which included an extra early earthing-up and a deeper grubbing, produced apparent small decreases for low dressings of sulphate of ammonia, but smaller increases for the heavy dressings. In spite of the exceptionally low standard error (1.8 per cent. of the mean yield per treatment as compared with an average of 7.5 per cent. for potato trials at Rothamsted¹), none of these yield differences for the cultivation comparisons reached the 5 per cent. level of statistical significance. The response to the sulphate of ammonia was, however, significant.

The British practices of inter-row tillage for potatoes have been influenced by a number of beliefs for which there is little or no scientific evidence. The several well-replicated field trials, described above, have established that under certain conditions in the U.S.A. the inter-row tillage of potatoes should be as shallow and as infrequent as possible: the limiting aims of such tillage should be the thorough destruction of weeds and the protection of the tubers by a moderate covering of earth. In America the workers have emphasized the root-damage to the potato plants which arises through stirring the soil more deeply or more frequently than necessary. The unexpected results of cultivation studies at Rothamsted and Cambridge and, in particular, the results of the Rothamsted studies of inter-row tillage in sugar-beet and kale, emphasize the possibility that the British potato crop also may not respond to such operations in the manner assumed by tradition. The only British data, a single year's results of an experiment in Scotland, show no response by potatoes to intensive cultivation.

5. *The Ottershaw Park Experiments on the Inter-row Tillage of Potatoes*

The author laid down at Ottershaw Park, Surrey, experiments covering the years 1937–9, inclusive, in order to obtain quantitative data on the response of main-crop potatoes to inter-row tillage for a light soil under British conditions. The experiments are only briefly summarized here, as a full description is given elsewhere.²

The hypotheses on which information was sought were: (a) that soil-stirring between plant rows is of direct benefit to the potato crop, in addition to its essential purpose of destroying weeds; (b) that the ridging-up of the plants is beneficial in addition to its main object of protecting the tubers from exposure to light and vermin.

¹ *School Science Review*, 1937, no. 74, 262.

² *J. Agric. Sci.*, 1941, 31.

These hypotheses were tested by three years of replicated plot experiments with main-crop potatoes, on a light sandy loam with a sand and gravel subsoil. Three principal treatments were compared:

- (N) ridging-up to a height of 6 in. with two preliminary grubblings;
- (F) from two to four preliminary grubblings in addition to the cultivations for (N);
- (U) or (S) control plots on which the soil was unstirred and the weeds were completely eliminated by surface scraping or hand-picking.

The three growing-seasons included spells of wet and of very dry weather, the rainfall varying from 18 to 30 in., and a sloping water-table maintained a continuous supply to at least one complete replication, while becoming too deep to affect the remainder. Under at least some of these varying conditions, these hypothetical benefits of ridging and grubbing, if they are real, should have become apparent. The mean yields for the three principal treatments are given in Table 2.

TABLE 2. *The Effect of Inter-Row Cultivation on Crop Yields*

Annual mean yields in tons per acre

<i>Treatment</i>	<i>F</i>	<i>N</i>	<i>U or (S)</i>	<i>Standard error</i>	<i>(F-U)</i>	<i>(F-U)% of U</i>
Number of cultivations . . .	4-6	2	0			
1937	12.36	10.37*	12.33	± 0.595	+0.03	+0.24%
1938	8.82	8.80	8.63	± 0.32	+0.19	+2.20%
1939	10.75	10.99	11.79	± 0.499	-1.04	-8.82%
3-yr. mean . . .	10.64	10.05	10.92		-0.28	-2.56%

* All plots considered in this table, except N of 1937, were weed-free.

The results do not support the hypothesis (a) set out above. The experiments of 1937 and 1938 were each replicated six times, and that of 1939 four times. Of the sixteen comparisons thus provided between the intensive (F) cultivations and the unstirred (U) or (S) controls, ten resulted in favour of the control treatment.

The percentage of ware potatoes (over the $1\frac{1}{2}$ in. riddle) was found for every plot in each of the three trials. It was not very variable and the trials were capable of detecting an effect of from 1 to 3 per cent. of the mean value. No result smaller than this would in any case be of much economic importance. The result of the experiments was that the percentage of ware was not affected by the cultivation treatments, provided weeds were absent. If the cultivations had proved beneficial to the plants, an increase in the proportion of large tubers could be confidently expected. That no such increase occurred in the three years is therefore further evidence against the original hypothesis.

Comparisons also carried out in one or more seasons included depth of grubbing; earliness of grubbing relative to the growth stage of the plant; effect of fertilizer-placing; and the effect of limited weed-competition when weeds were controlled only by the two cultivations of the

(N) treatment. Depth of grubbing had no apparent effect, and the times of grubbing little effect, either on the total yield or on the size of the tubers. A beneficial effect of placing of fertilizer in the bouts, as compared with harrowing it in on the flat, was strongly indicated, but was not conclusively demonstrated by the experiment. This fertilizer-placing significantly reduced the proportion of the ware potatoes spoiled by greening on the scraped control plots.

When the crop was cleaned only once before the final grubbing and ridging-up, weeds caused significant decreases in yield and in percentage of ware.

The potato crop in the Ottershaw experiments proved to be unexpectedly insensitive to inter-row tillage treatments. It is perhaps surprising that when in 1938 the intensive treatment was deliberately given over two weeks later, thereby inevitably causing additional root-pruning at a stage when the root-spread had reached the row centres, no very important reduction in yield was observed. Throughout the trials the crop seemed to be remarkably adaptable, and to be able to grow well under a wide variety of cultural practices.

The trials lay within the range of normal conditions under which potatoes are grown commercially on light land. Hence if the hypothetical benefits of inter-row cultivation are of general economic importance, they should have produced, in at least some part of the trials, positive yield increases more than sufficient to cover the cost of the additional cultivations. No such increases were in fact evident.

Only moderate dressings of dung and fertilizer were used; the plot yields were, however, good, all three being above the averages published annually for the British main-crop. This is important, since it shows that a high standard of yield may be maintained on light land, without deep or frequent soil stirring.

6. *Discussion on the Inter-row Tillage of Potatoes*

The mere demonstration of the ineffectiveness of intensive inter-row cultivation to increase the potato yield need not condemn these methods in actual farming practice. The practical grower could raise three further points requiring an answer before these extra cultivations can be profitably omitted, namely, (a) Is frequent grubbing and ridging-up the easiest way of keeping the land clean? (b) Do these extra cultivations give any indirect economic return by reducing the time spent on spraying and harvesting? (c) Do they benefit the succeeding crops in the rotation?

The unexpected susceptibility of the potato crop to competing weeds is in marked contrast to its lack of sensitivity to cultivation treatments. But it is possible that shallow sweeps or light spring-tine weeders, working to a depth of about 1 in., could replace deeper grubblings on light soils with a consequent appreciable saving of time and fuel.

The fitting of efficient haulm-lifting guards to the tractor and sprayer wheels, such as are already used with success in crops of laid corn, may prove a less expensive way of facilitating spraying than ridging-up.

The problem of harvesting appears to vary considerably with soil type.

The potato-lifting plough, fitted with a double set of lifting fingers, was used successfully for harvesting unridged guard-rows at Ottershaw Park, and this implement is much used in South Lincolnshire. The spinner undoubtedly works more easily with fairly high ridges, especially on heavy soils. The power-operated chain-elevator digger, a potato-harvesting implement used almost exclusively in some potato-growing areas of the U.S.A., appears particularly suited to modern tractor-cultivators. It is little known in England, but Hardenburg,¹ in observations on 254 fields of potatoes near New York, reports it to be capable of lifting crops ridged 2 in. high with only a little more damage than when the ridges were much higher. It may be possible to adapt the spinner to work with very low ridges on light soils, but while agricultural tradition favours high ridging, the majority of digging implements will continue to be designed for such conditions. In answer to point (b), therefore, if the potatoes are harvested by a lifting-plough or by a suitably designed digger, very slight ridging will suffice on light soils. It is important, however, that the ridging-up on such soils should be carried out at the minimum height determined by such practical harvesting requirements, and not be made as high as possible in an attempt to benefit the crop. Such misdirected higher ridging involves deeper preparatory grubbing, thereby causing unnecessary expenditure of power, with probably no effect on the crop beyond extra root-pruning.

The answer to point (c) must depend on the weed-population. If the potatoes are grown as the cleaning crop in a rotation, then certain perennial weeds may be best destroyed by frequent deep grubbing. Again, however, it is important that the grubbing on light soils be given with the definite object of weed-destruction, and that unproductive work in excess of these requirements be avoided.

These experimental results may prove inapplicable to heavy soils for the following reasons:

- (i) It appears probable that on heavy soils a loose mulch may serve a useful purpose by protecting the soil surface from baking into hard clods.
- (ii) It may not be possible, on stiff soils, to secure adequate protection for the tubers without cultivation at least 2-3 in. deep.
- (iii) Harvesting requirements may make higher ridges desirable. Stiff clay soils are, however, not well suited to potato-growing, as apart from the above considerations it is also difficult to separate the soil from the tubers.

Summary

1. Inter-row tillage of crops has been tested in the U.S.A. by many simple field trials with maize, and by several well-replicated modern trials with market-garden vegetables. The results have shown the maintenance of a soil mulch between plant-rows to be of little importance in the absence of weeds. The experiments emphasized the importance of weed-destruction. British evidence on inter-row tillage is scarce, but the data available confirm the findings of the American workers.

¹ *Amer. Potato J.*, 1934, **11**, 171.

2. Well-replicated American field trials on the inter-row tillage of potatoes have shown that, under the conditions tested, such tillage should be as shallow and as infrequent as is consistent with the thorough destruction of weeds. British data are limited to a single experiment, in which potatoes showed no significant response to inter-row tillage in the absence of weeds. This evidence is in sharp contrast to British farming tradition and practice for the potato crop, and further investigation is clearly necessary.

3. The experiments conducted at Ottershaw Park in the years 1937, 1938, and 1939 showed that for a well-drained sandy loam, under a considerable range of moisture-supply conditions, main-crop potatoes do not respond in the absence of weeds to ridging-up, or to deep or frequent inter-row grubblings, by any increase in yield or in the percentage of ware. The crop showed successful powers of adaptation to a range of contrasting inter-row tillage treatments.

4. The potato crop showed considerable sensitivity to weed-competition. This indicates that it is of great importance to maintain the crop in a weed-free condition.

5. Inter-row tillage operations on this type of soil should therefore be designed to destroy weeds and to provide moderate cover for the tubers. Intensification of such tillage beyond these limits is not of direct benefit to the plants.

6. These results may not be applicable to heavier soil types. It is probable, however, that they would hold good for most sandy loams. The results indicate the desirability of carrying out further experiments on contrasting British soil types, especially in the important potato-growing areas.

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STUDIES IN SOIL CULTIVATION

IX. THE EFFECT OF INTER-ROW TILLAGE ON THE YIELD OF POTATOES

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(With Two Text-figures)

THE inter-row tillage of a growing crop forms an intermediate part of a complex series of cultural operations. It is conditioned both by the efficiency of the seed-bed preparation and planting and by the need to secure favourable conditions for harvesting and for the subsequent crops of a rotation. Further, much confusion exists between the cultivation practices of successful growers and the various attempted explanations for these practices. The author has given elsewhere (1941) a general discussion of the published experiments on the effect of inter-row tillage on various crops. The experiments described in this paper were designed primarily to see if the main object of the inter-row tillage of the potato crop was weed suppression, or if the fine loose mulch which was produced as a by-product of this method of weed control had any beneficial effect on the growth of the crop.

Three primary comparisons were made in these experiments. In one the weeds were removed without any appreciable mulch being produced, either by hand-pulling or by a very shallow scraping of the surface soil. In the second, the weeds were destroyed by repeatedly grubbing in between the rows, thus maintaining a fine tilth. The third followed the usual farming practice of the neighbourhood, which consisted of a preliminary grubbing at an early date and then a second grubbing followed immediately by the earthing-up of the plants. The main object of the second grubbing was to obtain sufficient loose soil for the bouting plough to form good ridges easily.

A second object of the experiments was to find the effect of surface mulching on the moisture content of the soil, but owing to pressure of work this could only be investigated in 1937, the first year of the full experiments. In the course of this investigation, however, the depth of the water-table was found to fall from an average depth of 3 ft. 6 in. at

one end of the area to about 10 ft. at the other, and in the first two years the effect of the depth of the water-table on crop yield was measured.

All the grubbing cultivations were carried out with an "Auto-culto" motor hoe. Three "duck-foot" tines were used for the earlier grubblings and a central one with a narrow plain chisel point on either side for the later ones. These chisel point tines were moved in towards the centre of the row as the season progressed. Guards of bent iron were fitted over parts of the hoe to avoid haulm damage in the later operations.

In 1938 and 1939 the motor hoe was fitted with a device for keeping the weeds down without mulching the surface soil. The device consisted of three sweeps which scraped the surface soil without loosening it appreciably. The centre sweep was V-shaped and 12 in. across, while the side sweeps were set to work as near as possible to the plant. These two outer sweeps moved the shallow layer of earth loosened from the surface to the sides of the rows where it helped to protect the tubers from sunburn. At first a trailing wheel was fitted to ensure that the sweeps did not disturb more than the first $\frac{1}{2}$ in. of the soil surface, but this was later discarded as the operator found no difficulty in keeping them on the surface.

The experiments described here were all carried out at Ottershaw Park, Surrey. The soil is formed from the Bagshot Sands and contains up to 19 % clay in the top foot, much less in the second foot, and mainly sand and gravel below that. The field in which the experiments were carried out had been old pasture ley which was ploughed up in 1935 and sown to kale which failed. The area had become extremely foul with creeping thistle and other weeds when the land was laid down to this 4-year series of experiments.

The 1936 experiment was purely preliminary and was laid down with the dual object of cleaning the ground and of evolving a suitable technique for the cultivation experiments. Two ploughings were given, and with the second subsoiling was carried out across strips of the field. Only one-third of the plots was cropped. Three cultivation treatments, five grubblings, one grubbing and a hand-weeded control treatment were given. In the very wet growing season of 1936, however, the thistles got out of hand, and the latter two series of plots had to be cleared at intervals, instead of being kept continuously clean. They gave approximately equal yields while the plots kept clean by five grubblings showed a 14 % increase. It was not possible to ascertain how far this was due to weed destruction as compared with other factors. There was no sign of response to the subsoiling.

THE 1937 EXPERIMENT

The same area as used in 1936 received autumn cleaning after the potatoes, but a wet spring delayed ploughing in 1937 until 8 April. Although the subsoiling had shown no effect in 1936, it was an operation firmly believed in by neighbouring farmers, so that three of the six columns of experimental plots were subsoiled.

Plan of experiment.

Thirty-six plots, arranged in a special Latin square of which three rows, selected at random, were subsoiled.

Half of the plots carried "Majestic" potatoes while the remaining half were uncropped.

Three cultivation treatments were carried out.

Cultivation treatments.

F. Four cultivations; a loose 3 in. mulch was maintained, and earthing-up was thorough.

N. Two cultivations; given to destroy weeds.

U. No cultivations; weeds controlled by careful hand-picking, supplemented by weed-killer on the blank plots.

Ploughing treatments.

S. Shallow ploughing-in of farmyard manure, 5 in. only.

D. Subsoiling a further 9 in.; otherwise as for S.

Details of planting.

The plots were approximately $\frac{1}{100}$ acre. Potato plots carried four rows 32 in. apart, of which only the two centre rows were weighed.

Size of tubers.

All the potatoes were passed over a $1\frac{1}{2}$ in. riddle, and the chats and wares weighed separately for each plot.

The yields of the different treatments are given in Table 1.

The plots four times grubbed and then ridged up showed no difference, as compared with the uncultivated weed-free control plots, in total yield or percentage ware. Thus the inter-row cultivations appear to have benefited the crop only by destroying the weeds and not by maintaining a loose tilth.

The importance of this weed destruction is indicated by the small but significant decrease in the percentage of ware tubers, and the suggestion, although only on the 7 % level of significance, of a larger decrease in the

Table 1. *Total yield and percentage ware of potatoes (1937)*

(a) Effect of frequency of inter-row cultivation					
No. of grubblings	None		Two		Four
Total yield in tons/acre	12.33		10.37		12.36
Increase		- 1.93		+ 1.99	
% ware	91.79		88.11		89.80
Increase		- 3.68		+ 1.69	
					Standard error
					± 0.595
					± 0.842
					± 0.88
					± 1.24
(b) Effect of subsoiling					
	Shallow ploughing without subsoiling	Shallow ploughing with subsoiling	Difference due to subsoiling		Standard error
Total yield	12.23	11.10	- 1.13		± 1.78
% ware	90.87	88.93	- 1.94		± 2.51

total yield where the weeds were checked by two grubblings only. These grubblings, given at times when the weeds could best be destroyed, prevented them from establishing any obvious appearance of competition with the potato haulm. This suggests that even comparatively small weeds, growing thickly, can compete seriously with the potato crop. The weed effects thus disguised any possible effect of ridging-up on yield.

The subsoil ploughing had no effect on the crop yield or size of tubers. It is difficult indeed to imagine how it could be expected to benefit the crop on such a well-drained sandy soil, unless the sand were too compact to permit root penetration. In digging the pits for water-table location, however, roots under normal ploughing were observed well below the 12 in. level reached by the subsoiler. It must be considered probable that the subsoiling, if fairly and critically tested, would have proved to be equally ineffective on the similar neighbouring soils in which this identical equipment had been working for some years.

THE 1938 EXPERIMENT

The failure of the potato crop to respond to inter-row grubbing and ridging in 1937 was either an unusual lapse from the characteristics traditionally ascribed to the crop, or else it was an indication that the potato is not in reality sensitive to intensive tillage under these conditions of soil and climate. The results could only be interpreted therefore in the light of further trials, and the main comparisons were therefore repeated in the design for the 1938 experiments.

The intensive cultivations in 1937 had continued rather late in the growth stage of the plant. To investigate the manner in which this might affect the results, the "F" treatment was duplicated in 1938, one set of

cultivations (F) beginning 14 days and ending 19 days before the other (F'). The "N" or simple grubbing-and-ridging treatment was duplicated in order to estimate separately the effects of the ridging and weed competition. On one set of plots (N) the weeds were controlled by two cultivations only, the plants being otherwise left to smother the weeds, while in the duplicate set (N'), given the same tillage, all weeds were removed with a minimum of soil disturbance, by careful hand-cleaning. The control treatment was also duplicated, in an attempt to secure weed destruction with minimum soil disturbance, by a more practical means than hand-picking. The experiment was laid down on a contiguous strip of the field used for the 1937 trials.

Plan of experiment.

A 6 × 6 Latin square was used, all plots being cropped with Doone Star potatoes.

Details of cultivation treatments.

F. Six cultivations, the last one being followed immediately by boutting-up.

F'. Six cultivations given from 15 to 19 days later than those for the "F" plots, and finally boutting-up.

N. Two cultivations given when necessary for weed destruction. The second was followed by boutting-up.

N'. Cultivations as for "N" plots, but weeds eliminated by hand-picking.

U. No mulching, but the weeds were removed by surface scraping with flat sweeps on the motor-hoe set by a trailing wheel to a depth of about $\frac{1}{2}$ in.

U'. No cultivations. The plots were kept completely free from weeds by hand-picking.

All plots were hand-hoed lightly between the plants before boutting-up.

Trampling of the soil was avoided during hand-picking of weeds by the use of long light boards.

Size of plots.

Approximately $\frac{1}{160}$ acre.

Planting details.

Four rows constituted a plot, of which only the middle two were measured. Rows were set 36 in. apart in order to minimize haulm damage from cultivations. Plant intervals were reduced to 1 ft. to compensate for increased row width.

Size of tubers.

All potatoes were lifted by hand and passed over a $1\frac{1}{2}$ in. riddle. The weight of tubers affected by sunburning or greening was also noted for each plot.

The results of this experiment are given in Table 2.

Table 2. *Total yields and percentage ware for 1938 experiment*

Treatment symbol No. of cultivations Details of cultivations	Total yield (expressed as tons per acre)						Standard errors
	F 6 Given early	F' 6 Given later	N 2 Some weeds	N' 2 Weeds hand- picked $\frac{1}{2}$ in.	U 0 Surface scraped $\frac{1}{2}$ in.	U' 0 Weeds hand- picked	
Total yields in tons per acre	8.94	8.70	6.83	8.80	8.76	8.49	± 0.44
Mean for six cultivations 8.82; mean for no cultivations 8.63; difference 0.19 ± 0.31 ; difference due to weeds, N'-N, 1.96 ± 0.63 .							
Ware % of total yield	92.7	92.4	84.9	92.1	92.5	90.4	± 0.79
Differences: N'-N = 7.2 (significant); U-U' = 2.1 (not significant)							± 1.12

The most striking feature of these results is that the means for the four weed-free, motor-hoed treatments F, F', N', and U showed no apparent response by the crop to the three very different tillage treatments. The differences in the means, both of yield and of percentage ware, are not only well within the margin of error, but are also, from the viewpoint of the practical grower, negligible in comparison with the differences in the cost of cultivation. Of the six comparisons between F and U cultivations in the six blocks three favour each treatment. The almost identical mean yields of the N and U plots show that, in the absence of weeds, two grubblings and a ridging-up of the plants served no purpose other than the protection of the tubers by ridging. The ridges do not thus appear to have provided an environment more encouraging to the crop than the flat culture of the plots receiving three surface scrapings only. The four extra grubblings produced little or no effect, although there is a suggestion of a slight response in yield when these are given earlier; but the difference of 2.8 % in yield between F and F' is not statistically established, and may be due to chance.

The use of flat sweeps on the motor-hoe for removing weeds at the surface level proved to be a better method than the hand-picking. The necessity of hand-weeding when the soil was too wet, in the early part of the season, caused excessive packing, in spite of the fact that long boards were laid between the rows on these occasions in order to minimize trampling.

The competition of small weeds produced significant decreases both in yield and in percentage of ware, as is shown by the comparison between treatments N and N'. The actual percentage reduction of yield on the N plots below the mean of the other five treatments for each of the six blocks was:

Block ...	I	II	III	IV	V	VI
% reduction in yield	11.3	18.4	27.1	23.5	43.4	0.9
% reduction in ware	2.2	-1.4	10.7	12.6	13.6	2.3

Only on the plot in block V was the weed infestation really severe, the weeds being principally bind-weed (*Convolvulus avensis*), groundsel (*Senecio vulgaris*) and young goose-foot or fat-hen (*Chenopodium album*). The reduction of yield by nearly one-half on the weedy plot of block V would not have been surprising if the plot had remained weedy throughout the season, but it was thoroughly cleaned twice before ridging-up on 6 July, and it remained clean thereafter. The deleterious effect of the weeds in the early stages of the crop growth, before the cultivations were given, must therefore have accounted for most of the yield reduction. Substantial reductions also resulted on four of the remaining five plots on which, judged by eye, the weeds would not appear capable of harming the plants. There were indications, discussed below, that competition for moisture was the principal reason for weed damage.

Tubers greened by exposure to light. All tubers showing signs of greening were carefully picked out and weighed separately for each plot:

Treatment	F	F'	N	N'	U	U'
% of greened tubers	0.59	0.52	0.88	0.43	1.46	3.25
Means for cultivations	0.56 %		0.65 %		2.35 %	
	(bouted)		(bouted)		(flat)	

Although the plots grown on the flat produced a relatively high proportion of greened tubers, the slight cover provided by scraping (U) with flat hoes reduced this wastage to less than 1.5 %. This is still nearly three times the proportion wasted by the bouted-up plots on which little more than half of 1 % were spoiled. It might be further reduced if the scraping sweeps were designed to cast more soil sideways.

THE 1939 EXPERIMENT

The field trials for two consecutive years had shown no effective response by the potato crop to ridging-up, with or without frequent grubbing, when all weeds were removed by other means. Practical growers, with whom the results were discussed, suggested that "It takes deep working to do any good". The depth of 3 in., previously used as

typical of local practice on light soils, was varied in 1939 to include grubbing both 3 and 6 in. deep.

For the surface cultivations of the control plots, wide flat sweeps on the motor-hoe had proved very satisfactory in 1938, and these were used for all of the control plots in 1939. It had been found that although irregularities of the soil surface caused a very slight occasional mulching effect on the first operation, this became less with successive scrapings. The sweeps were arranged to move any soil loosened by scraping towards the plants. The blades were sharpened in order to minimize soil disturbance, thus providing a more effective comparison with the ridging and grubbing treatments.

A possible effect of fertilizer placing on the extent of root-pruning damage was investigated by splitting the plots for two methods of fertilizer application. It was considered that a concentration of fertilizer near to those roots which are out of reach of the grubbing implements might be an advantage, and the plots were therefore split to compare the sowing of fertilizer in the planting furrows with broadcasting followed by harrowing in on the flat.

The striking effects observed in 1938 of the competition of small weeds with the potato crop were considered worthy of further investigation in 1939. An attempt was made to extend this study by direct sampling for an estimate of weed density.

The trials for 1938 had shown only a suggestion of the expected advantage of early, as compared with later, grubblings. This comparison was therefore dropped in favour of a study of depth of working. The frequent grubblings were begun as soon as the plant rows were clearly visible, the necessity of avoiding accidental pre-emergence damage being doubly important under the conditions of an experiment.

The standard error for the total yield data in the two previous years had been rather high (12.5 and 12.9 % respectively), although such variation is not unusual in plot trials. The rather small size of the plots (approximately $\frac{1}{100}$ acre in both years) was a possible contributory factor, and in 1939 the plot area was increased to $\frac{1}{70}$ acre.

Details of the experimental design.

Four randomized blocks of eight plots each were used. This gave four replications of the seven treatments and of one dummy comparison.

Details of treatments.

F 6. Five cultivations to a depth of 6 in., the last being followed immediately by boutting-up.

F 3. Five cultivations to a depth of 3 in., followed immediately by boutting-up.

Nc 6. Two 6-in. cultivations only. Plots kept weed-free by additional surface scraping. Boutted-up after second cultivation.

Nc 3. Two 3-in. cultivations only. Otherwise as for Nc 6.

Nw 6. Two 6-in. cultivations only, but with no additional weed control. Boutted-up immediately after the second cultivation.

Nw 3. Two 3-in. cultivations only. Otherwise as for Nw 6.

S. No soil-stirring cultivations, but plots were kept weed-free by scraping with flat hoes, working at about $\frac{1}{2}$ -in.

S. Ditto (dummy comparison).

Fertilizer application.

All plots were halved for the application of fertilizer by two methods. The half-plots were harvested separately.

H. Fertilizer harrowed-in on the flat before drawing out the furrows for planting.

B. Fertilizer applied in the furrows during planting.

Size of plots.

The harvested area of each plot consisted of four 20 yd. rows 32 in. apart. All plots were separated by guard rows, and outer plots were protected by double guard rows. The harvested area of each plot was thus approximately $\frac{1}{70}$ acre.

Planting details.

Variety: Majestic (certified Ross-shire seed).

Spacing: Rows were 32 in. apart, and sets were 15 in. apart.

Boutting-up.

This was done by a double mould-board hand plough, drawn by a tractor on pneumatic tyres.

The equipment was taken throughout the crop, the plough being raised to slide along the surface when traversing S plots.

Some haulm-crushing by the tractor tyres occurred, but this was shared equally by all plots, and the following weeks of intermittent rain brought rapid recovery, removing most traces of the damage. A little of the haulm on the N and F plots remained buried.

Site of the experimental plots.

The much larger area of the 1939 experiment was arranged to cover most of the combined sites of the two previous experiments. A con-

trasting soil type would have been an advantage, but no suitable alternative site was available. As in previous years, the blocks were arranged at right angles to the direction of slope of the water-table, and the long narrow plots were paralleled to the slope. Thus any effect on yield of differing moisture supply from the water-table was eliminated in the statistical analysis of the results. The results of this experiment are given in Table 4.

Table 4. *Total yields and percentage ware for 1939 experiment*

(1) Treatment means								
	S	F 6	F 3	Nc 6	Nc 3	Nw 6	Nw 3	S.E.
Yield (tons/acre)	11.78	10.51	10.98	11.05	10.93	8.93	10.74	0.70
% ware	90.6	90.7	90.7	91.1	90.0	89.1	90.2	0.49
Nc 6-Nw 6. Yield difference 2.12 ± 1.00 ; % ware difference = 2.0 ± 0.69								
(2) Effect of frequency of cultivation								
	S No cultivations	F Five cultivations	Nc Two cultivations (weed-free)	Nw Two cultivations (weedy)	S.E.			
Yield	11.78	10.75	10.99	9.67	± 0.50			
% ware	90.6	90.7	90.5	89.7	± 0.35			
(3) Effect of depth of cultivation (weed-free plots only)								
	$\frac{1}{2}$ in. surface scraping	3 in. cultivations	6 in. cultivations	S.E.				
Yield	11.78	10.88	10.78	± 0.50				
% ware	90.64	90.3	90.9	± 0.35				

Where weeds were removed, there were again no signs of any response by the potato crop, either to ridging-up with two preliminary grubblings, or to ridging with five preliminary grubblings as compared with surface scraping. Of the four comparisons between the F and S treatments provided by the four blocks, three were in favour of S. The small difference in the mean yields, below statistical significance, even suggested some reduction in yield from the ridging-up and a slight further reduction from additional grubblings. The percentage ware was very uniform. There is a similar suggestion of a slight further decrease in yield for the 6 in., as compared with the 3 in. grubbing. The percentage ware shows, if anything, a trace of the opposite effect. These differences are not, however, established and must be considered to be due to the various factors of experimental error.

Weed competition was less intense in 1939, since the area had been partially cleaned by the preceding potato crops. The weedy plots gave, however, a significantly lower mean yield than the remaining weed-free plots, though almost the whole lowering was due to the Nw 6 plots, i.e. those cultivated to 6 in. The probable reason was that the two grubblings

on the Nw 3 plots were done on 19 June and 1 July, while on the Nw 6 plots the first grubbing was delayed until 25 June. The difference of yield of 2.12 tons per acre was produced by comparatively small weeds which nowhere dominated the haulm. The weeds were destroyed twice during the growing season and were largely smothered by the haulm after the second cleaning. There was a severe drought in June, and this result probably illustrates the effect of weed cleaning at a critical stage of the competition between the crop and the weeds for their water supply.

AN ESTIMATE OF WEED DENSITY

On 18 June, just before the first grubbing of the Nw 3 plots, an estimate was made of the weight of weeds on each of the Nw plots. A light wooden frame was constructed to enclose an area of $\frac{1}{2}$ m. sq. This was placed centrally in the space between the rows, and the weeds within the frame were pulled. The roots were cut off to ground level by scissors, and the plants were immediately weighed on a spring balance. The whole operation was thus carried out fairly rapidly in dry sunny weather, the operator wearing tennis shoes in order to minimize trampling of the soil. This trampling on the dry soil had a negligible effect. One random sample was taken in this manner in each of the five inter-row spaces of each subplot. The sample means thus obtained were observed to agree closely with an eye estimate of the weediness of the plots.

Fig. 1 shows the potato yields in lb. per half plot plotted against the weed weight on 18 June for both the Nw 3 and Nw 6 series. There is a high positive correlation between the weed density and plot yield instead of a negative correlation as would be expected. The more fertile plots grew both more weeds and more potatoes than the less fertile.

Two pairs of (N) plots in each block differed only in the presence or absence of weeds. Their differences in yield are set out below, with the corresponding weed density estimates. A negative sign indicates an apparent increase of yield due to weeds.

Block	Yield difference in lb. Nc 3-Nw 3	Weed density in gm./sq. m.	Yield difference in lb. Nc 6-Nw 6	Weed density in gm./sq. m.
I	- 2	184	+ 96	220
II	- 29	400	+ 79	488
III	- 10	844	+ 96	600
IV	+ 46	404	+ 4	1188
Mean yield per plot in lb.	176		162	

The correlation ($r=0.263$) is insignificant for eight pairs of values. The shallow-grubbed plots, grubbed in mid-June, show no average reduction in yield from the weed competition. The deep-grubbed plots, grubbed a week later, show considerable yield reductions, but these are

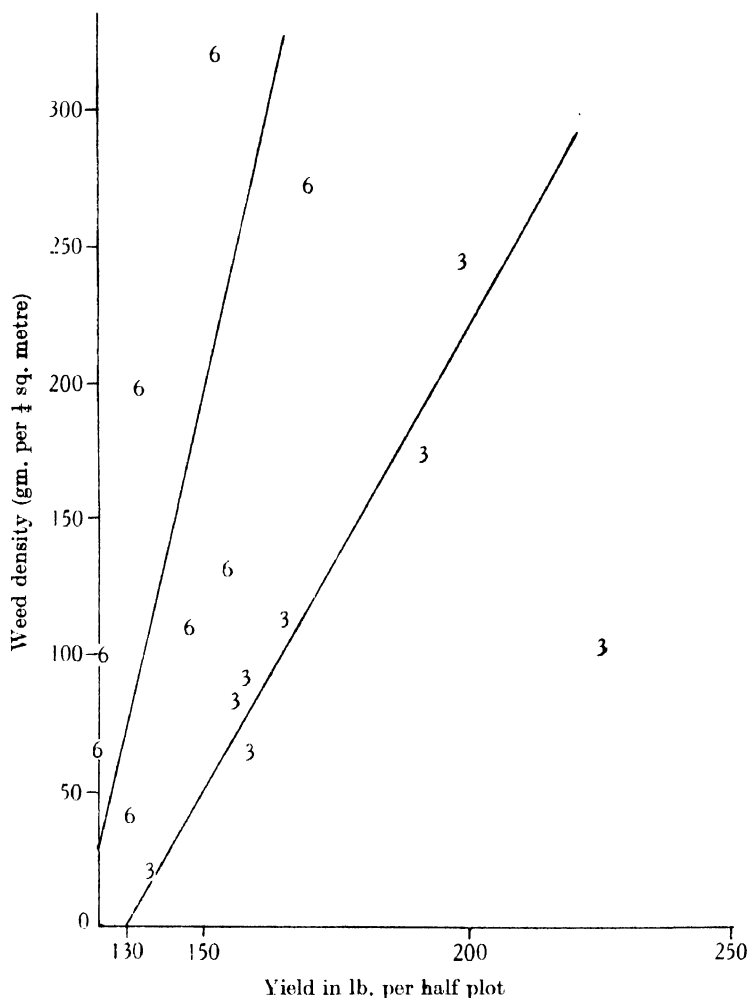


Fig. 1. Weed density and crop yield.

6 refers to Nw 6 plots; 3 refers to Nw 3 plots

not significantly related to the weed density as shown by the sample. This probably indicates that the method of sampling the fresh weight of weeds does not give any satisfactory measure of the intensity of their competition with the crop.

THE EFFECTS OF FERTILIZER PLACING

The effect of placing the fertilizer in the bouts before planting as compared with harrowing it in before drawing out the bouts was small. There was a small apparent gain of 2.5 % in yield due to placement which was far below statistical significance.

The fertilizer was, however, subjected to heavy rain for a week while lying in the furrows, on a very well-drained sandy soil. This would be expected to produce a decrease in yield as compared with the half-plots on which the fertilizer had been protected from rain by harrowing in. The result, however, was that six out of the eight treatments, and the experiment as a whole, showed slight suggestions of a gain. This suggests that there may be a real advantage in this method of placing the fertilizer as near as possible to the sets, although the experiment does not produce any reliable evidence for this.

The fertilizer placing had no effect on the size of the tubers. The very small differences in the means shown by percentage ware are all well within the margin of experimental error.

The fertilizer placement did not appear to give any marked effect on any of the treatments, though there was a suggestion, well below statistical significance, that the effect of weed competition was less noticeable on the yield when the fertilizer was placed closer to the plants. As a corollary, the weight of weeds harvested on 18 June was smaller on the half-plots in which the fertilizer was placed than on those in which it was harrowed in, but again the difference of weed weight was well below statistical significance.

The placement of the fertilizer had, however, a definite effect on the percentage of the ware potatoes in the scraped series S spoilt by greening. Spoilt ware on the half-plots in which the fertilizer was placed amounted to 2.5 % as against 4.5 % on the plots in which it was harrowed in, and this 2 % reduction was statistically significant.

THE EFFECT OF A SURFACE MULCH ON THE
MOISTURE CONTENT OF THE SOIL

In the 1937 experiment, eighteen plots, forming half of the Latin square, were given the same cultivation treatments as the potato plots, but were kept bare and clean of weeds by applying a light dressing of arsenious weed killer. The moisture content of these plots was determined three times, on 18 July, 2 and 28 August, during a spell of dry

weather, the July and August rainfalls being only 1.28 and 1.13 in. The moisture content of each plot was determined on three bulked 2 ft. samples which were thoroughly mixed, quartered and dried at 110° C. for 24 hr. The difference in moisture content between the frequently mulched (F series) and the unmulched (U series) plots was 0.03–0.54 and –0.28 % on the three sampling dates respectively giving a mean loss of water due to mulching of –0.26 %, which is well within experimental error.

These determinations were repeated in 1938. The spring and summer were hot and dry, the June and July rainfalls being 0.75 and 1.17 in. Samples were taken on 16 and 29 June and 17 July midway between the rows of the plants down to a depth of 18 in., and were dried at 110° C. for 24 hr. as before. The difference in moisture content between the frequently mulched (F and F' series) and unmulched (U and U' series) plots was 0.79, 0.32 and –0.51 % on the three sampling dates respectively, giving an apparent mean gain of moisture content of 0.22 % due to mulching. All the differences, however, were just within experimental error.

Only one set of moisture determinations was made in 1939 on 5 July after a long dry spell in June, and after this the weather broke. The methods used were similar to those used in 1938, except that bouting had already been done on 1 July, so that the depth of sampling was 21 in. in the bouts, 15 in. in the furrow and 18 in. on the flat. The plots (F 3) on which a 3 in. mulch was maintained continuously had a moisture content 0.13 % less than the unmulched (S) plots. This was well within experimental error.

The experiments on the effect of a surface mulch, in the absence of weeds, on the mean moisture content of the first 18 in. or 24 in. of the soil showed that it gave a mean increase of moisture content of 0.22 % in 1938 and a mean decrease of 0.26 and 0.13 % in 1937 and 1939 over the unmulched soil, giving a mean reduction of 0.06 % for the 3 years. The mulch thus had absolutely no effect on the moisture content of the first 18 in. of soil.

Mulching may, however, cause a definite loss of water as compared with clean weeded land if the water-table is near the surface. In 1938 one block, block VI, had a water-table at a mean depth of just less than 3 ft. 6 in., while the mean depth on the other five blocks varied from about 4 ft. 9 in. to 9 ft. 6 in. The high water-table kept the plots of this block wetter than the other blocks, and mulching caused a pronounced lowering of moisture content, instead of the gain which the traditional

capillary hypothesis would predict. The results of this sampling, which was done on 17 July, were:

	Depth of water-table	Moisture content		Mulch-unmulched
		Mulched (F')	Unmulched (U')	
Block VI	About 3 ft. 6 in.	9.80	13.16	- 3.36
Blocks I-V	Below 4 ft. 6 in.	8.09	7.99	+ 0.10

This may only have been a chance effect, for it was not shown at all clearly in 1937. Three separate samplings were taken on land carrying no crop, and the mean results were:

	Approximate mean depth of water-table	Moisture content		Mulched-unmulched
		Mulched (F)	Unmulched (U)	
Block VI	About 3 ft. 3 in.	13.60	14.92	- 1.32
Block V	About 3 ft. 6 in.	13.64	13.16	0.48
Blocks I-IV	Below 4 ft.	11.09	11.28	- 0.19

The results of the three separate samplings were perfectly concordant, the difference mulched-unmulched being:

Sampling date	18. vii. 37	2. viii. 37	28. viii. 37
Block VI	- 1.26	- 1.99	- 0.70
Block V	0.50	0.43	0.51
Blocks I-IV	0.24	- 0.43	- 0.37

A possible explanation of this difference is that in both 1937 and 1938 the two plots concerned in block VI were contiguous, while they were not in block V, and as five water-table heights were taken just outside the experimental area, it is possible the apparent anomaly of block V is due to the water-table being higher under the mulched plot than under the unmulched.

Mulching does, however, conserve moisture if the comparison is made between clean mulched land and weedy land. In 1938 the difference in moisture content on block V between the weed-free plots and the plots of series N, which only received two grubblings and no hand-weeding, was a loss of 1.44 and 1.33 % of moisture content due to weeds on 16 and 29 June respectively, which lie well outside the limits of experimental error. The yield, as already noted on p. 218, was 43 % lower on this weedy plot than on the rest of the block, and it is possible that this great reduction was due to the competition of weeds with the potato plants for the strictly limited supply of water. Further evidence can be found for this explanation since the yield of potatoes per block seemed to be correlated with the mean moisture content of each block on 18 July in 1937 and on 17 July in 1938, as is shown in Fig. 2. It is

interesting to note that the block which fell off the regression line in 1938 was very close to the area occupied by the block which fell off in 1937.

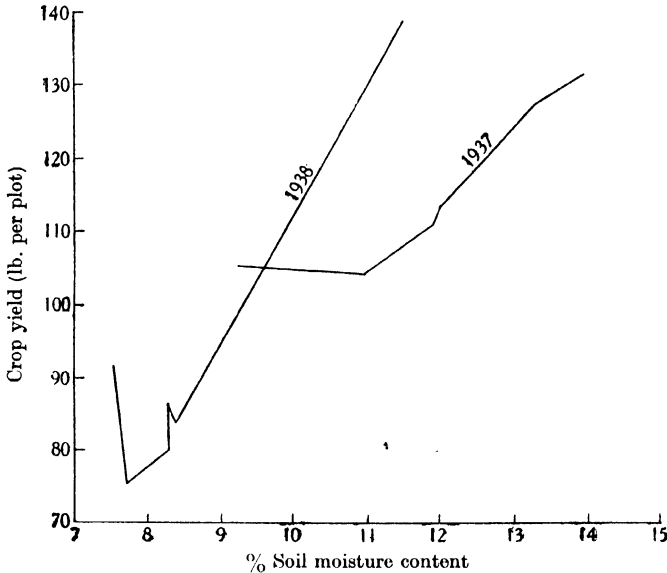


Fig. 2. The relation between crop yield and the moisture content of the first 18 in. of soil. (Block means.)

THE EFFECT OF THE HEIGHT OF THE GROUND-WATER-TABLE ON THE MOISTURE CONTENT OF THE SOIL

The height of the water-table was observed in several plots in both 1937 and 1938, and it was found to vary between about 3 and 10 ft. In both years moisture contents were available, in 1937 there were three separate samplings on the bare plots of the first 2 ft. of soil and in 1938 only one of the first 18 in. of soil, but since it was taken from the bottom of the furrow between two ridges, it really extended from a depth of 2 ft. up to a depth of 6 in. The moisture contents of these samples were:

Year	Water-table	
	Between 3-4 ft.	Below 4 ft.
1937	14.0	11.2
1938	11.5	8.0

DISCUSSION OF RESULTS

The yield of potatoes in these trials was definitely above the annually published national average for main crop potatoes in two of the years and just above in the third, although only very moderate dressings of dung and fertilizers were used. Hence a high standard of yield was obtained on light land without deep or frequent soil stirring.

The potato crop proved unexpectedly insensitive to inter-row tillage. It was, in fact, surprisingly so in 1938, when one set of intensive grubbing which was given a fortnight later than usual, caused no significant reduction of yield although it must inevitably have caused more extensive root pruning at a stage when root spreading had reached the centre of the row. Throughout the trials the crop seemed to be remarkably adaptable and to grow well under a wide variety of cultural practices.

The susceptibility of the potato yield to competing weeds was in marked contrast to its lack of sensitivity to cultivation treatments. It is possible that this susceptibility may have been due to some specific limiting factor, for which both crop and weeds compete, and if so this factor was probably shortage of water rather than shortage of any particular nutrient.

This demonstration of the negative result of cultivation to produce significant increases of yield would not satisfy the practical grower if these cultivations are the only way of keeping the weed population low or if they reduce the time spent on spraying and harvesting or if they benefit the succeeding crops in the rotation.

These results suggest that shallow sweeps or light spring-time weeders could replace grubbing and boutings on light soils with considerable saving of time and fuel, if they were used fairly frequently when the crop was still young. A shallow cultivation at about 1 to 1½ in. appears to be adequate for weed destruction on such soils.

Inter-row cultivations are often the easiest way to obtain a sufficiency of fine tilth for bouting-up, and so long as bouting-up is an essential part of potato culture, many of these tillages will probably be done for this reason alone. Bouting-up is probably done for three reasons. First it reduces the damage the sprayer does to the haulms when it goes through the crop. Fitting efficient haulm lifting guards to the tractor and sprayer wheels, such as are already used with success in laid corn, would probably prove a more efficient way of reducing haulm damage than bouting-up. Secondly, the bouts reduce greening of the tubers, but very low ridges are all that are needed for this purpose. Thirdly, the existing potato harvesting

machinery works much better on ridged than on flat land. But this limitation can be overcome, on light land at any rate. The potato-lifting plough, fitted with a double set of lifting fingers, was used successfully for harvesting unridged guard-rows at Ottershaw Park, and this implement is much used in South Lincolnshire. The spinner undoubtedly works more easily with fairly high ridges, especially on heavy soils. The power-operated chain-elevator digger, a potato harvesting implement used almost exclusively in some potato-growing areas of the U.S.A., appears particularly suited to modern tractor conditions. It is little known in England, but Hardenburg (1934), in observations on 254 fields of potatoes near New York, reports it to be capable of lifting crops ridged 2 in. high with only a little more damage than when the ridges were much higher. These results suggest that in light soils the grubbing and ridging policy should aim at the minimum height of ridge to which the harvesting equipment can be adapted.

The effect of intensive cultivation of the potato crop on succeeding crops depends on the weed population. If the potatoes are grown as the cleaning crop in a rotation, then certain perennial weeds may best be destroyed by frequent deep grubbing. Again, however, it is important that the grubbing on light soils be given with the definite object of weed destruction and that unproductive work in excess of these requirements should be avoided.

These experimental results may not be applicable to heavy soils. In the first place a loose mulch on heavy soils may prevent the soil surface from baking into hard clods and thus facilitate the destruction of the weeds. It may not be possible to obtain sufficient fine soil to protect the tubers without cultivating the soil 2-3 in. deep. Harvesting requirements may also make higher ridges desirable.

Soil moisture content has been shown by modern studies to be a highly variable quantity, and a more extensive programme of sampling would have been desirable, if minor effects of the 3 in. mulch on the moisture of the first 2 ft. of soil were to be reliably detected. The samplings were, however, sufficiently intensive to detect any moisture-conservation effect of the mulch great enough to be of economically important benefit to the crop. No such effect occurred in three seasons of widely varying rainfall. The range of water-table depths was such that capillary rise of soil moisture was important for some complete replications, and unimportant for others. Thus the experiments included conditions representative of the principal range of water-table situations likely to occur on light arable soils. For none of these conditions was the mulch effective.

An explanation of this ineffectiveness of the mulch to conserve moisture is offered by the relation observed between the moisture content of the upper soil and the depth of the water-table. The results of research into the movement of water in soils show that important capillary movement of soil moisture is to be expected only where a continuous supply from a water-table is occurring, and then only through a strictly limited distance. This distance, on the sandy soil studied, proved to be no more than 4 ft. Where the water-table lay deeper, any water lost by evaporation from the upper soil could not be replaced from below. No capillary rise therefore existed, thus rendering the mulch ineffective. The lack of effect where capillary rise did exist, may possibly be explained by the rapid replacement from below of any water lost from the unmulched plots, so that on these plots the layer dried by evaporation was no deeper than that of the comparable 3 in. soil mulch. The demonstration of the limit of capillary rise in a natural soil under field conditions is rarely possible, and the result is, therefore, of considerable interest.

SUMMARY

The experiments conducted at Ottershaw Park in the years 1937, 1938 and 1939 indicate that for a well-drained sandy loam, under a considerable range of moisture-supply conditions, main-crop potatoes do not respond in the absence of weeds to ridging-up, or to deep or frequent inter-row grubblings, by any increase in yield or in the percentage of ware. The crop showed successful powers of adaptation to a range of contrasting inter-row tillage treatments.

The potato crop showed considerable sensitivity to weed competition in the early stages of growth. This indicates that it is of great importance to maintain the crop in a weed-free condition during this early period.

Inter-row tillage operations on this type of soil should, therefore, be designed to destroy weeds and to provide moderate cover for the tubers. Intensification of such tillage beyond these limits is not of direct benefit to the plants.

No moisture-conservation effect of any importance was produced by a 3 in. soil mulch during dry weather, even when the water-table lay within 4 ft. from the surface.

A significant upward movement of water from the water-table to the surface soil was observed, but this was limited to a vertical distance of approximately 4 ft. for the soil type described. Beyond this limit the surface soil was apparently unaffected by the depth of the water-table.

These results may not be applicable to heavier soil types. It is probable, however, that they would hold good for most sandy loams.

The results indicate the desirability of carrying out further experiments on contrasting British soil types, especially in the important potato-growing areas.

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STUDIES IN SOIL CULTIVATION

X. THE RESULTS OF A SIX-YEAR CULTIVATION EXPERIMENT

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THE influence of the tilth of a seed-bed produced by different agricultural implements on the growth and yield of different crops has been studied here by Keen and his co-workers for a number of years. The results are given in the previous nine papers of this series. Up to 1934 only experiments lasting for one or two years had been carried out, but in 1933 a six-year cultivation rotation experiment was started on the Rothamsted Farm which was discontinued after the harvest of 1939. Russell & Keen (1938) and Russell & Mehta (1938) have discussed all the experimental data accumulated on this farm up to 1936, that is, including the results of the first three years of this rotation experiment. The purpose of this paper is to discuss the six years' results.

The general object of the experiment was to find out how far different methods of preparing a seed-bed affected the yield of the crops and the weediness of the land. The three methods chosen consisted in ploughing the land and following by whatever harrowings and rollings were considered necessary (treatment P), grubbing the land with a tractor cultivator followed by harrowings and rollings (treatment G) and obtaining a seed-bed in a single operation by using a small rototiller (treatment R). Each of these primary operations of ploughing, grubbing or rototilling was carried out at two depths of 8 and 4 in., called the deep and shallow treatments, but in order to work to 8 in. with the grubber and rototiller it was necessary to go over the land twice.

The reasons for choosing these three treatments were as follows: The plough cuts a definite furrow and inverts it. This usually, though not necessarily, causes some comminution of the soil surface and so helps to form the necessary seed-bed tilth. But further comminution and consolidation by harrows and rolls is still necessary. The tractor-drawn grubber, or cultivator, breaks up the soil surface without inversion. In

most years, however, it does not leave the land in a suitable condition for a seed-bed and more working with harrows is usually needed. The rototiller, on the other hand, produces a seed-bed in one operation that is usually quite fine enough though it would often be considered too loose. The rototiller achieves this by having tines which, by rotating about a horizontal axis, dig and break up the soil. The experiment was on three adjacent parcels of land which were cropped in the three-course rotation, wheat, mangolds and barley, each parcel carrying a different crop. Each parcel was subdivided into two, by a 40 link (8.05 m.) path down the centre, and each of these were again divided into two. These four divisions of each parcel will be called blocks. Each block was divided into twelve long narrow plots, 139.8 links by 11 links (or 28.12 by 2.21 m.) with a headland on the central path. Twelve different treatments were used, which were the twelve possible combinations of ploughing, rototilling and grubbing, carried out either deep or shallow and dressed with either calcium cyanamide or nitrochalk as the source of nitrogen. The nitrogen was applied to the wheat as a top dressing of 0.3 cwt./acre of N, to the barley a dressing of 0.2 cwt./acre of N was applied at least a week before drilling, and to the mangolds the cyanamide, at the rate of 0.6 cwt./acre of N, was applied at least a week before drilling and half the nitrochalk was applied at this time and the other half at singling. The mangolds also received a basal dressing of 0.75 cwt./acre of P_2O_5 as superphosphate and 1.0 cwt./acre of K_2O as muriate of potash. No other fertilizers were applied.

The four blocks in each parcel were used as follows. On two of the blocks the same cultivation treatment was repeated every year without change, and these blocks form the Continuous Series. On the other two blocks the cultivation treatments rotate. Each plot that is worked deep one year is worked shallow the next, each plot receives nitrochalk for two years and then calcium cyanamide for two years, and on one block the cultivation rotation follows the order plough, rototiller, grubber, and is called rotation A, and on the other the order is plough, grubber, rototiller and is called rotation B. These two cultivation rotations were included so that if, in the following year, there were any residual effects of rototilling or grubbing the land instead of ploughing it, they could be determined.

During the course of the six years the following additions and modifications were made to the cultivation treatments. From 1933 to the harvest year 1936-7 the three primary cultivations of ploughing, rototilling and grubbing were done on the same day, with the exception

of 1936-7 when the wheat ploughed plots were done ten days before the rototilled or grubbed plots. After this date each cultivation was carried out when the farm manager judged it would be most suitable. This resulted in the rototilling being done just before drilling and the ploughing and grubbing being done several days previously.

Trouble was experienced from the very beginning with weeds, and in particular the young plant suffered. To overcome this the wheat stubble was shallow ploughed in the autumn of 1935, and in each subsequent year preparatory to the main spring cultivations for the mangolds. In the last year of the experiment the barley stubble was also shallow ploughed and harrowed preparatory to the main autumn cultivations for the wheat. In the harvest years of 1934-5 and 1936-7 the ploughed and grubbed plots of the barley break were ploughed and grubbed respectively in the winter directly after the mangolds had been carted off, and again in spring just before the seed-bed was prepared.

The general way of preparing the seed-bed after the main cultivations had been done was to harrow and roll the ploughed and grubbed plots once or twice in the direction of cultivation and then to cross-harrow and roll the whole break once across before drilling. The experimental design had, however, a very grave defect in those seasons when it was difficult to obtain a seed-bed, for it did not allow of cross-cultivations being given to only the ploughed and the grubbed plots which needed them and not to the rototilled plots which did not.¹ Only one really difficult season was encountered however. In 1937 the following cultivations had to be given to all plots to obtain a suitable seed-bed for mangolds: spring-tine harrowed across once, lengthways once, and then tractor rolled and drag harrowed once lengthways, across once and then lengthways once, so that the rototilled plots had perforce to have extensive subsequent cultivations to allow a reasonable seed-bed to be prepared on the ploughed and grubbed plots. After this year rototilling was usually deferred until just before drilling, so that the harrows which were used to prepare a suitable seed-bed on the ploughed and grubbed plots could traverse the rototilled plots before they were cultivated.

Various cleaning operations were carried out in the growing crop. The winter wheat was usually harrowed and rolled in March or April and the barley was usually rolled in April or May. In 1934 and 1938 both the barley and wheat plots, and in 1936 the barley plots were hand-

¹ This defect in design was inevitable, as the farm does not possess any close-coupled implements; cross-cultivation with normally hitched implements would have required impossibly wide headlands along the sides of the plots.

hoed in the beginning of May, though only on the wheat plots in 1938 did there seem to be many weeds. If these hoeings had any effect on the crop growth, they would probably tend to reduce the differences due to the various cultivations. The mangold plots were regularly horse-hoed and sometimes hand-hoed as well.

The only other modification that had to be made was in March 1936. The winter wheat had failed, so the whole break was spring-tine harrowed and resown with spring wheat.

The investigation, which was, in part, designed to find out how to conduct long-term cultivation experiments, showed up clearly the importance of the already mentioned fundamental defect, that it was not possible to cross-cultivate selected plots only. This was particularly serious on the grubbed plots, for a much fairer comparison would have been made if the deep grubbed plots had been worked both ways by the grubber and not only lengthways. It also resulted in the rototilled plots having to receive unwanted cross-cultivations which probably were slightly harmful to the tilth.

A further limitation was that neither the grubber nor the rototiller could bury dung, so that none could be added during the experiment. This is not a fundamental limitation of this experiment as there is no evidence that dung is essential for these crops during a six-year experiment on this soil, but the dressings of artificials given were probably too low, bearing in mind the absence of dung, and this is probably the cause of a deterioration of yield that set in, particularly on the barley plots, towards the end of the experiment.

THE EFFECT OF THE TREATMENTS ON THE CROP YIELDS

The yields of each plot have been printed in the *Annual Reports of Rothamsted Experimental Station* for the years 1934–8, and the individual 1939 results will be printed in the next *Report*.

In this section only short summaries of the treatment effects on crop yield will be given, and their form will sometimes be determined by the fact that, since the crop rotation is a three-year one, any appreciable variations due to soil heterogeneity will be eliminated from comparisons between mean yields or mean treatment effects for the first three years and the second three years.

In some tables standard errors have been given to the mean effects of some treatments. If the means only involved plots of the Continuous

Series the variance, i.e. the square of the standard error, of the mean was obtained by dividing the sum of the residual variances of the plot yields over the period of years involved by the suitable factor. This could not be done for treatment means involving plots of the Rotating Series because the residual variances of the plot yields per year cannot be calculated as there was no true replication of these treatments. Approximate standard errors for such means have been calculated for some tables by assuming that, in each year, the variance per plot was the same on the Rotating as on the Continuous Series.

THE LEVEL OF YIELDS

Table 1 gives the mean yields of the three crops for each of the six years, together with the yields of wheat and barley on neighbouring manurial rotation experiments for a comparison.

Table 1. *Level of yields, 1934-9*

Experiment year	Wheat (grain in cwt./acre)		Barley (grain in cwt./acre)		Mangolds (roots in tons/acre) Cultivation
	Cultivation	Two neigh- bouring	Cultivation	Three neigh- bouring	
1934	23.4	27.0	26.2	26.1	35.9
1935	21.2	22.2	35.2	33.9	20.4
1936	21.3	15.7	25.7	27.5	20.7
1937	15.0	16.8	15.0	20.0	19.4
1938	11.8	30.2	16.9	31.0	13.7
1939	25.8	23.2	19.5	33.2	24.6
Mean 1934-6	22.0	21.6	29.0	29.2	25.7
1937-9	17.5	23.4	17.1	28.1	19.2

The yield of wheat on this experiment was similar to that on neighbouring experiments in all years except 1938, when for some unexplained reason there was only a thin plant of wheat which did not tiller well and which looked miserable all through the season. The low yield in 1937 was due to the season and not to the particular conditions on the experimental plots. There has, therefore, been no marked deterioration in the yield of wheat on this experiment compared with neighbouring experiments.

The barley yields, on the other hand, show a marked deterioration of yield in the second three-year period compared with the first, which is not reflected in the yields of barley elsewhere on the farm. This can be seen from Table 1, for whereas the mean barley yields on the neigh-

bouring rotation experiments were 29·2 and 28·1 cwt./acre for the three-year periods 1934-6 and 1937-9 respectively, those on this cultivation experiment were 29·0 and 17·1 cwt./acre, giving a mean drop in yield of about 11 cwt./acre.

There have been no other mangold experiments in the immediate vicinity of this one, though in 1936 and 1937 mean yields of 25 and 21 tons/acre were obtained elsewhere on the farm. The mean yield of 19·2 tons/acre in the second three-year period is lower than 25·7 tons/acre in the first due to the good crop in 1934 and the bad crop in 1938, but owing to this lack of other mangold experiments it is not possible to say definitely how far this reduction is due to a general deterioration of the soil fertility in this experiment or how far it is due to general seasonal factors.

The results of neighbouring sugar-beet experiments suggest that while a part of the lowering is probably due to seasonal effects—for 1934 was also a good and 1938 a bad year—yet a considerable part is due to a lowering of soil fertility presumably due to an inadequate supply of artificials considering that no farmyard manure is supplied. This is brought out in the following table which gives the mean yield of sugar beet in tons/acre in two manurial rotation experiments, one on each side of the cultivation experiment (third column) and in one-year experiments on different fields that were in non-experimental farming for two to three years previously (fourth column):

Crop Experiment	Mangolds Cultivation Rotation	Sugar-beet Manurial Rotation	Sugar-beet One year
Mean yield in tons/acre for the period			
1934-6					25·7	10·6	12·9
1937-9					19·2	7·7	11·7
Percentage reduction in the second three-year period					25	28	10

The yields on the sugar-beet rotational experiments, neither of which had any dung added, follow those of the mangold crop very closely from year to year and show the same apparent deterioration of yield. As the one-year experiments also show an apparent deterioration of yield, part of this is presumably due to seasonal factors. But part may also be due to deterioration, as the mean dressing of sulphate of ammonia in the rotational experiments is only 0·3 cwt. of nitrogen per year, which is much lower than most of the levels used in the majority of the one-year experiments. Thus although no rigorous deductions can be made as to how much, if any, deterioration took place, it is possible that the mangold

yields did show a loss of about 15 % of crop due to an inadequate supply of nitrogen.

Hence the main conclusion to be drawn from these comparisons is that probably the level of manuring on this cultivation experiment was inadequate to maintain for six years the yields of barley and mangolds, though it was adequate for the wheat crop. This result will be discussed in more detail later on in this paper.

COMPARISON OF NITROCHALK AND CALCIUM CYANAMIDE AS THE NITROGENOUS FERTILIZER

A comparison was made between nitrochalk and cyanamide as the form of the nitrogenous fertilizer, since there was a possibility that cyanamide might depress somewhat the growth of weeds. In this event we should expect the plots receiving cyanamide to give a higher mean crop yield or to deteriorate rather slower than those receiving nitrochalk.

The experimental results actually showed that, over the six years, the mean yields on the nitrochalk plots were higher than on the cyanamide plots by the following amounts:

	cwt./acre		cwt./acre		tons/acre
Wheat grain	0.4	Barley grain	0.4	Mangold roots	0.4
Wheat straw	1.6	Barley straw	0.2	Mangold tops	0.3

The only figure calling for any comment is that for the wheat straw, which every year was definitely higher on the nitrochalk plots. This result can be explained in the following way. Watson (1939) found that at Rothamsted a top dressing of sulphate of ammonia applied in March gave a higher straw yield than if applied in April or later, though the yield of grain was hardly affected. The nitrochalk and cyanamide were usually applied in March or early April, and if the cyanamide became available more slowly than the nitrochalk it should be equivalent to a later dressing of available nitrogen and should therefore cause a depression in the straw yield, as found.

There is no evidence that there is any difference of behaviour of nitrochalk and cyanamide in the second three years of the experiment as compared with the first three years. Hence cyanamide has no accumulative beneficial or harmful effect as compared with nitrochalk.

The experiment suggests, therefore, that there is no difference in the response of the crops to cyanamide and nitrochalk unless the time of application of the nitrogen is important.

THE YIELD ON THE PLOUGHED PLOTS

There are four sets of ploughed plots whose yields are worth separating out, the deep and the shallow ploughed plots on the Continuous and on the Rotating Series. The mean yields of these four treatments for the six years are given in Table 2.

Table 2. *Mean crop yields on the ploughed plots*

	Wheat (cwt./acre)		Barley (cwt./acre)		Mangolds (tons/acre)	
	Grain	Straw	Grain	Straw	Roots	Tops
Continuous: Deep	22.65	34.94	23.71	29.15	24.54	5.72
Shallow	22.82	34.74	24.59	29.47	23.81	5.63
Rotating: Deep	22.28	33.48	23.92	29.02	24.04	5.68
Shallow	21.45	32.42	23.32	28.04	22.72	5.45
Continuous minus Rotating	0.87	1.89	0.53	0.78	0.80	0.11
Deep minus Shallow	0.33	0.63	-0.14	0.33	1.03	0.16
Approx. standard error*	0.36	0.68	0.43	0.50	0.44	0.10

* This refers only to the Deep minus Shallow comparison and not to the Continuous minus Rotating, whose standard error is probably considerably larger.

The main results that emerge from this table are that the effect of depth of ploughing is negligible, except possibly for the mangold crop, and that the yield is slightly higher on the Continuous than on the Rotating Series, implying that there may have been a small reduction of yield on those ploughed plots that were rototilled and grubbed in the two previous years. The mean yields of the ploughed plots on rotation A were about the same as on rotation B, so that the harmful residual effect due to using these two types of cultivator, if it existed, did not depend on whether the three-year cultivation rotation was grubber-rototiller-plough or rototiller-grubber-plough. This possible harmful residual effect will be discussed more fully in a later section.

The mean yields of the crops on the ploughed plots of the Continuous Series for the first and second three-year periods of the experiment are given in Table 3.

Table 3. *Mean yields on the Continuously ploughed plots*

	Wheat (cwt./acre)		Barley (cwt./acre)		Mangolds (tons/acre)	
	Grain	Straw	Grain	Straw	Roots	Tops
Mean yield: 1934-6	23.83	38.66	30.11	36.13	27.88	5.46
1936-9	21.63	31.03	18.19	22.48	20.47	5.89
Reduction of yield	2.20	7.63	11.92	13.65	7.41	-0.43

This table shows the reduction of yield on the continuously ploughed plots only in contradistinction to Table 1 which is for the whole experiment. The differences between the two tables are small for barley and mangolds, but rather larger for wheat.

Table 4. *The crop response to depth of ploughing in each three-year period*

Series ...	Wheat grain (cwt./acre)		Barley grain (cwt./acre)		Mangold roots (tons/acre)	
	C	R	C	R	C	R
1934-6:						
Deep minus Shallow	-0.77	0.39	0.18	0.50	1.70	0.10
Standard error	0.79		0.99		1.11	
1937-9:						
Deep minus Shallow	0.43	1.29	-1.95	0.71	-0.24	2.54
Standard error	0.66		0.69		0.59	

Series C, continuous; Series R, rotating.

Table 4 shows that this reduction in yield is about the same on the deep as on the shallow ploughed plots of the Continuous Series. But there does seem to have been a response by wheat and mangolds to deep ploughing on the Rotating Series in the second three-year period, probably because the ploughing was following on land that, having been worked mainly by the grubber or rototiller for the preceding two years, was more weedy than the ploughed plots on the Continuous Series. This should not apply to the barley crop, which in fact does not show this response, as it follows the mangold crop which left the land clean, since it was hoed several times. Hence this beneficial effect of deep ploughing is probably due to its having a greater depressing effect on the weed population than the shallow ploughing.

Thus over the six years of the experiment there is no evidence that ploughing below a 4 in. depth confers any benefit to the crop that is reflected in its yield unless the land is dirty, when it may be advantageous to plough deeper. This result is in accord with the general run of experimental results on this farm.

COMPARISON OF THE PLOUGH AND ROTOTILLER

The wheat and mangold yields were definitely lower on the rototilled than on the ploughed plots over the six-year period, while the barley yields were about the same on the deep rototilled plots and a little down on the shallow, as is shown in Table 5. For all the crops, except mangold tops, the yields were higher on the deep rototilled plots than on the

Table 5. *Mean yield of the ploughed and rototilled plots*

	Wheat (cwt./acre)		Barley (cwt./acre)		Mangolds (tons/acre)	
	Grain	Straw	Grain	Straw	Roots	Tops
Mean ploughed, P	22.30	33.90	23.88	28.92	23.78	5.62
Rototilled deep, RD	18.84	30.10	24.26	28.29	22.44	5.19
Rototilled shallow, RS	17.80	28.84	22.54	26.78	21.21	5.26
Rototilled: Continuous Series	18.05	29.17	23.28	27.76	21.98	5.22
Rotating Series	18.60	29.57	23.52	27.31	21.67	5.23
P - RD	3.46	3.80	-0.38	0.63	1.34	0.43
P - RS	4.50	5.26	1.34	2.14	2.57	0.36
Approx. standard error of differences	0.32	0.59	0.37	0.43	0.39	0.08

shallow. This result is probably not due to the mere increased depth of working, as the results of the ploughed plots showed that depth was not important. There is the possibility that the greater depth was in fact important for the particular kind of tilth produced by the rototiller and, as will be shown in the next section, by the grubber. A more probable explanation is that, because deep rototillage involved going over the land twice, it is due to the finer tilth and to the better burial of weeds and weed seeds on the deep than on the shallow tilled plots, both these differences being confirmed by Russell & Mehta's (1938) findings.

The deterioration of yield on the rototilled plots in the second over the first three-year period is given in Table 6. The table shows that the rate of deterioration of the barley and mangold yields appears to be independent of the cultivation treatment, except that the yield of mangolds on the continuously shallow rototilled plots appears to deteriorate rather slower than on the rest of the experiment.

Table 6. *The deterioration of yield on the rototilled plots*

	Wheat [*] (cwt./acre)		Barley (cwt./acre)		Mangolds (tons/acre)	
	Grain	Straw	Grain	Straw	Roots	Tops
Ploughed Continuous Series	2.20	7.63	11.92	13.65	7.41	-0.43
Continuous rototilled deep	5.53	10.65	11.70	13.90	6.55	-0.02
Continuous rototilled shallow	6.05	10.93	11.60	14.08	4.34	-0.08
Rotating rototilled: Deep	4.81	7.70	12.79	13.57	6.61	-0.29
Shallow	4.70	8.81	11.63	12.72	6.04	0.07

The results for wheat are in marked contrast. The rototilled plots have definitely deteriorated more than the ploughed plots, and as Table 7 shows this occurs on each of the four main treatments. This table also shows that the deep, and twice, rototilled plots of the Rotating

Table 7. *Decrease in yield of wheat grain in cwt./acre on the rototilled below the ploughed plots*

	Rotating Series		Continuous Series	
	Deep	Shallow	Deep	Shallow
1934-6	0.99	2.10	2.32	3.47
1937-9	4.95	5.03	6.25	6.72

Series yielded almost as well as the ploughed for the first three years. The beneficial effect of the deeper rototillage was not affected by the deterioration of yield, since compared with the shallow rototilled plots it produced mean increases of yield of 0.4 and 0.9 cwt. of grain per acre on the Continuous and 1.5 and 1.4 cwt./acre on the Rotating Series over the periods 1934-6 and 1937-9 respectively. Hence it is difficult to argue that the wheat yields are lower on the rototilled than on the ploughed plots because the tilth on the rototilled plots is too fine for winter wheat, as it is precisely those plots most likely to possess a deep fine loose tilth that give the smallest reduction of yield in the early years of the experiment.

COMPARISON OF THE PLOUGH AND GRUBBER

The mean yields of the crops over the six-year period are given in Table 8. The grubbed plots yield less in all cases than the ploughed, though the reduction for the deep grubbed is not very large for barley or mangolds. Again in all cases the shallow (and once) grubbed plots do not yield as well as the deep (and twice) grubbed ones. The continuously grubbed plots yield rather less than those grubbed in rotation though the difference is negligible for wheat and for mangold tops.

Table 8. *Mean yield of the ploughed and the grubbed plots*

	Wheat (cwt./acre)		Barley (cwt./acre)		Mangolds (tons/acre)	
	Grain	Straw	Grain	Straw	Roots	Tops
Mean ploughed, P	22.30	33.90	23.88	28.92	23.78	5.62
Grubbed: Deep, GD	18.81	29.04	22.87	27.40	21.90	5.22
Shallow, GS	17.74	28.07	21.83	26.53	21.06	5.31
Grubbed: Continuous Series	18.20	28.39	21.62	26.06	20.83	5.20
Rotating Series	18.35	28.72	22.87	27.87	22.13	5.34
P - GD	3.49	4.86	1.21	1.52	1.88	0.40
P - GS	4.46	5.83	2.05	2.39	2.72	0.31
Approx. standard error of differences	0.32	0.59	0.37	0.43	0.39	0.08

The deterioration of yield on the grubbed plots in the second three-year period compared with the first is given in Table 9. The deeper

Table 9. *Deterioration of yield on the grubbed plots*

	Wheat (cwt./acre)		Barley (cwt./acre)		Mangolds (tons/acre)	
	Grain	Straw	Grain	Straw	Roots	Tops
Ploughed Continuous Series	2.20	7.63	11.92	13.65	7.41	-0.43
Continuously grubbed: Deep	6.57	11.98	12.83	13.48	7.56	-0.35
Shallow	6.33	9.65	10.52	12.60	5.26	-0.34
Grubbed Rotating Series: Deep	5.90	8.68	12.96	14.29	7.83	-0.42
Shallow	4.55	7.73	12.36	14.50	6.21	-0.45

grubbed plots seem to deteriorate rather more rapidly than the shallow, particularly on the Continuous Series, though this effect is only appreciable for wheat straw, barley grain and mangold roots. This is due to the level of yields on the deep grubbed plots of the Continuous Series having fallen to the level of the shallow grubbed plots of this series in the second three-year period, as is shown in Table 10. In the last three-

Table 10. *Decrease of yield of the Continuous shallow grubbed below the Continuous deep grubbed plots*

	Wheat (cwt./acre)		Barley (cwt./acre)		Mangolds (tons/acre)	
	Grain	Straw	Grain	Straw	Roots	Tops
1934-6	1.27	2.63	2.08	1.70	2.27	-0.15
1937-9	1.02	0.30	-0.23	0.82	-0.03	-0.14

year period only wheat grain shows any benefit from deep as compared with shallow grubbing, and this is hardly significant as the three annual values of this benefit were 1.40, 2.40 and -0.75 cwt./acre. The mean yields for these three years were 9.5, 11.0 and 24.3 cwt./acre so that deep grubbing appeared to be of benefit when the yields were very low.

Table 9, in conjunction with Table 6, shows that the rates of deterioration of the barley and the mangold yields are the same for the plough, the rototiller and the grubber, so that this deterioration cannot be attributed to the method of cultivation. Presumably it is due to an inadequate supply of artificial fertilizers. Wheat, however, shows a greater deterioration on the grubbed than on the ploughed plots, as did the rototilled plots. When the results are analysed out in further detail, as in Table 11, it is seen that they show exactly the same behaviour as the rototilled, namely that in the first three years the wheat yield on the deep (and twice) grubbed plots was nearly the same as on the ploughed

plots but that in the second three-year period they had sunk to much lower values.

Table 11. *Decrease in the yield of wheat grain in cwt./acre of the grubbed below the ploughed plots*

	Rotating Series		Continuous Series	
	Deep	Shallow	Deep	Shallow
1934-6	0.91	2.21	1.40	3.43
1937-9	5.95	5.00	6.37	6.95

COMPARISON OF THE ROTOTILLED AND THE GRUBBED PLOTS

The mean differences in yield between the rototilled and the grubbed plots are given in Table 12. There is a general tendency for the grubbed plots to have a slightly lower yield than the rototilled, and this is still true when more detailed comparisons than those between full means are

Table 12. *Increased yield of the rototilled over the grubbed plots*

	Wheat (cwt./acre)		Barley (cwt./acre)		Mangolds (tons/acre)	
	Grain	Straw	Grain	Straw	Roots	Tops
1934-6	-0.24	0.81	1.03	0.48	-0.07	0.11
1937-9	0.33	0.82	1.27	0.66	0.76	-0.20
Mean	0.09	0.82	1.15	0.57	0.35	-0.04
Approx. standard error of mean	0.37	0.69	0.43	0.50	0.44	0.10

considered. For the first three years wheat grain, but not straw, did rather better on the grubbed than rototilled plots, particularly on the deep plots of the Continuous Series. Barley definitely did better on the rototilled plots, while the mangolds sometimes did better on the rototilled and sometimes on the grubbed. The main result of this comparison is, however, the smallness of the differences between these two cultivation treatments.

THE RESIDUAL EFFECTS OF THE ROTOTILLER AND GRUBBER

The possible existence of harmful residual effects on the crop yield due to using the rototiller or the grubber instead of the plough has already been mentioned in the section dealing with the crop yield on the ploughed plots. This point will now be examined in more detail. There were three different cultivation series in this experiment, the first containing those plots that always received the same method of cultivations, the Continuous Series, the second those plots in which the method of

cultivation followed the three-year cultivation rotation—plough-rototiller-grubber, called rotation A, and the third those plots cultivated in the order plough-grubber-rototiller—called rotation B. Thus the ploughed plots on the two Rotating Series differ from those on the Continuous Series in that the former were not ploughed in the two previous years while the latter were. If the grubbed or rototilled land was in a less favourable condition than the ploughed at the end of the cropping season, it is possible that it will carry a poorer crop in the following season if both are treated the same. Under these conditions the crop yield ought to be higher on the ploughed plots of the Continuous Series which have been ploughed all the time than on the ploughed plots of the Rotating Series, which were not ploughed the two previous years. Table 2 has already shown that grubbing and rototilling appear to have small harmful residual effects in comparison with the plough, but these differences are not precisely determined as they have been obtained from differences of yield between plots in different blocks and not between plots in the same block. A more detailed analysis of the data into two three-year periods does not give any additional confirmation of this result as the results of the comparisons become erratic.

A further method of examining the question whether grubbing or rototilling have harmful residual effects is to compare the mean yield of the rototilled plots of rotation A, which were ploughed the previous year, with those of rotation B which were grubbed the previous year and with those of the Continuous Series which were always rototilled. If the previous year's rototilling or grubbing left any harmful effects in the succeeding year that the ploughing did not, then the yield should be higher on rotation A than on rotation B or on the Continuous Series. In the same way the yield of the grubbed plots on rotation B which were ploughed the previous year should be higher than those on rotation A which were rototilled the previous year or on the Continuous Series

Table 13. *The harmful residual effects of not ploughing the land (method II)*

	Wheat grain (cwt./acre)		Barley grain (cwt./acre)		Mangold roots (tons/acre)	
	Rot. 1- Rot. 2	Rot. 1- Cont.	Rot. 1- Rot. 2	Rot. 1- Cont.	Rot. 1- Rot. 2	Rot. 1- Cont.
Rototiller:						
Deep	1.0	1.7	-0.1	0.1	1.9	0.2
Shallow	3.6 (a)	1.8 (a)	1.7	1.0	2.1	1.1
Grubber:						
Deep	-0.4	-0.4	-0.1	2.0	0.0	0.3
Shallow	-0.5	-0.1	2.1 (b)	3.3	0.0	3.1

which were always grubbed. The five-year means of these differences are given in Table 13. In this table rotation 1 is rotation A for the rototilled and rotation B for the grubbed series and rotation 2 is the other rotation. The wheat entries for the shallow rototiller series, marked (a) are almost entirely due to very high yields on the two plots of rotation A in 1937, which gave differences of 12.3 and 9.2 cwt./acre respectively for that year. The entry marked (b) for the barley yield under shallow grubbing is entirely due to very low yields on the two plots of rotation A in 1938, which gave a difference of 10.0 cwt./acre for that year. Cochran (1939) gave a rather more accurate method than this for estimating the residual effects of grubbing or rototilling the land, but the results of this more elaborate calculation do not differ appreciably from the simpler rotation 1-rotation 2 comparison.

The interpretation of Table 13 is not quite straightforward, since for the deep rototilled plots the difference rotation 1-rotation 2 really gives the residual effect of the shallow grubber compared with the shallow plough, for these are the treatments the plots received the previous year, while rotation 1-Continuous gives the residual effect of deep rototiller compared with the shallow plough. But the general impression one gets from the table is that the residual effects, if they exist, are small and very erratic.

There is still a third method of testing for any appreciable residual effects by comparing the difference of yield between all those ploughed and, say, those rototilled plots on the Rotating Series which were grubbed the previous year with the difference between the ploughed and rototilled plots on the Continuous Series. The first difference is what Cochran (1939) called the direct effect of the rototiller in comparison with the plough, since the previous year's cultivation was the same for each treatment and can be calculated rather more accurately than is described here. The second difference he called the continuous effect as it contains all harmful residuals resulting from rototilling the land year after year instead of ploughing it year after year. The difference between these represents the sum of any harmful residual effects due to the rototillings in the previous years.

Table 14 gives these harmful residual effects both for the full five years available and for the last three, when they should be most noticeable. This table again gives no evidence that there are any appreciable residual effects of not ploughing the seed-bed.

The conclusion reached is, therefore, that under the experimental conditions employed, there appeared to be no appreciable residual effects

Table 14. *The harmful residual effects of not ploughing the land (Method III)*

	Wheat grain (cwt./acre)		Barley grain (cwt./acre)		Mangold roots (tons/acre)	
	1935-9	1937-9	1935-9	1937-9	1935-9	1937-9
Plough minus rototiller:						
Deep	1.5	0.4	0.1	-1.0	-0.5	-1.7
Shallow	0.6	0.3	0.9	1.2	0.6	0.3
Plough minus grubber:						
• Deep	-0.8	-0.8	1.4	0.4	0.7	-0.7
Shallow	0.3	0.5	2.1	0.9	2.9	2.2

of grubbing or rototilling the land instead of ploughing it in the following year. But this conclusion is limited by the important experimental condition that all the plots were shallow ploughed as soon after the wheat was harvested as possible in preparation for the mangold crop.

MISCELLANEOUS OBSERVATIONS ON THE CROP GROWTH

A number of eye observations have been taken on the crop, mainly at harvest. The usual remarks for the wheat and barley are that the ploughed plots carry a taller plant ripening more evenly than the cultivated plots. The shallow cultivated plots usually carried a gappy plant and in a bad year one of very variable height and for barley containing many green ears at harvest. In general the ploughed plots carried the fewest and the shallow cultivated plots carried the most weeds. There was, however, no close connexion between total yield and general weediness during the growing season, since the deep cultivated plots sometimes gave the same crop yield but carried a larger weed population than the ploughed.

There were exceptions to these generalizations. Two blocks of the barley experiment in 1938 provide an example when they did not hold. There was a poor early stand on the ploughed and the grubbed plots and a better, but still only mediocre, stand on the rototilled plots. This was not due to weeds as the two blocks were still clean but may have been due either to differential bird damage or to poor tilth causing uneven germination. There were indications that the bare patches had a rougher tilth and were a little drier than where the barley was showing, but the differences were only small. At harvest all eight of the rototilled plots were described as having an even plant of uniformly ripe ears while only one of the eight ploughed plots was so described and none of the grubbed, though one had uniformly ripe ears. None of the rototilled

plots were described as weedy though several of the ploughed and grubbed were. In this case weediness was a result of the patchiness of the crop and not a cause, whereas normally it is at least a partial cause.

One other observation was made on the wheat crop in four out of the six years of the experiment. The wheat ears tended to be larger on the deep ploughed plots than on the shallow but the number of ears rather fewer. In three of these years the straw was noted as being taller and stronger on the deep ploughed than the shallow ploughed plots. These two effects were also noted on the deep grubbed plots in 1935.

Weeds were usually more in evidence in the early stages of growth on the non-ploughed mangold plots, particularly on those shallow tilled, though they could never dominate the crop for long as it was hoed at least once before and usually several times after singling. At the time of pulling all the plots were clean, the only exception being in 1936 when half the plots could not be properly hoed towards the end of the season with the consequence that they carried a strong weed population throughout the latter part of the growing season. These late weeds did not, however, affect the yield.

The number of mangold roots pulled per plot was counted every year, and except for 1935 was almost independent of cultivation treatment as is shown in Table 15. In 1935 the effect of treatment is marked,

Table 15. *Mean number of mangold roots harvested per plot**

(1934-9, omitting 1935.)

	Plough	Rototiller	Grubber
Deep	162	161	159
Shallow	164	159	160

* To obtain roots per acre multiply by 130.

Table 16. *Mean number of mangold roots harvested per plot in 1935*

	Continuous Series			Rotating Series		
	Plough	Rototiller	Grubber	Plough	Rototiller	Grubber
Deep	168	157	157	166	159	146
Shallow	181	140	103	173	149	155

as is shown in Table 16. The plant numbers were highest on the ploughed plots, and in the Continuous Series there was a considerable reduction on the shallow rototilled and a great reduction on the shallow grubbed plots. This result is probably due to the great variations in the weediness of the seed-beds produced by the various cultivation implements. Russell & Mehta (1938) showed that in 1934 there was a poorer apparent

germination of mangolds on the weedier plots than on the cleaner, but that it was sufficient on every plot for an almost even plant to be set at singling. In 1935 the seed-beds on the non-ploughed plots were weedy, and the shallow grubbed plots of the Continuous Series were particularly foul. Weeds seem to have caused a sufficiently large mortality among the young seedlings on some of these plots to prevent a full plant being set, and hence the large variations in plant number. To overcome this trouble in future years it was decided to plough the whole of the wheat stubble to a 4 in. depth in the autumn of 1935 and every subsequent year in preparation for the mangold seed-beds. This had the desired cleaning effect so that in the subsequent years a fairly even plant was set at singling as can be seen from Table 15.

The roots were sometimes rather smaller on the rototilled and the grubbed plots than on the ploughed, but the differences were not large, as is shown in Table 17.

Table 17. *Mean root weight of mangolds in lb.*

	Plough	Rototiller	Grubber
1934-6: Deep	2.96	2.91	2.85
Shallow	2.75	2.76	2.83
Mean	2.86	2.83	2.84
1937-9: Deep	2.19	1.96	1.93
Shallow	2.06	1.95	1.91
Mean	2.13	1.96	1.92

One other comment was made about the mangolds in each of the last three years. At lifting time the leaves were predominantly dark green on the deep ploughed plots, a lighter green on the shallow ploughed becoming yellowish or yellow on the rototilled and the grubbed, this being more marked on those shallow than those deep tilled.

THE RELATIVE INFLUENCE OF SEED-BED TILTH AND WEEDS ON CROP YIELD

The question that arises from these experiments is how far the differences in crop development on the various plots are due to differences in the tilth of the seed-bed at the time of sowing and how far to differences in weed infestation. No definite answers can be given to this question since direct estimates of seed-bed tilth and weediness during the growing season are not available. There is no question that the very low yields on some plots was due to them being foul with weeds, but the question of greater importance is whether the average losses of crop due

to grubbing or rototilling are due to the greater weed population they carry or to their giving a seed-bed having a poorer tilth.

There is one general consideration pointing to weeds being an important factor in causing this reduction in yield. The rototiller working shallow always seemed to prepare as good a seed-bed as the shallow plough treatment, yet the yields were usually lower and sometimes considerably lower while the weed infestations were often considerably higher. The rototiller working deep gave a deeper seed-bed which was probably only a little looser and a little finer than when working shallow, but it usually carried a larger crop with fewer weeds. Since the depth of ploughing did not affect the yield, it seems quite plausible to assume that it was the decreased weediness rather than the increased depth of seed-bed that was mainly responsible for the increased yield.

Turning to the crops individually, it was often noted that the wheat straw was rather shorter on the non-ploughed than on the ploughed plots. It is unlikely this would be a seed-bed effect as the crop is winter sown, but is much more likely to be due to weed competition for available nitrogen as suggested by Blackman & Templeman (1939), particularly since this was most noticeable at the end of the experiment when deterioration of yield due to insufficient manuring was probably setting in.

A second interesting point is that there was a definite tendency for the grain on the deep ploughed plots to be plumper and the straw taller but for the crop to be less uniform than on the shallow ploughed, so that the yield per acre was unaffected. It is possible that the plumper grain and the taller straw may have been due to the slightly greater freedom from weeds and that the greater unevenness of plant was due to the rather rougher and less uniform seed-bed on the deep than on the shallow ploughed plots.

There is a general belief that barley starts off much better if it has a fine seed-bed; this may be the reason why the deep rototilled plots gave as good a yield as the ploughed, although they carried a heavier weed population. Here tilth is almost certainly of some importance and this is probably borne out by the 1938 barley results already discussed. The evidence for the harmful effect of weeds is less clear than for wheat. In the first place the mean reduction of yield on the shallow grubbed plots, usually the weediest, below the ploughed plots is only 2 cwt./acre for the barley grain compared with $4\frac{1}{2}$ cwt./acre for the lighter wheat crop. But the barley followed the mangolds so weed competition may have been less severe.

Weeds early in the season, however, might easily set the plant back somewhat, thus making it less uniform in height and maturity at harvest than the cleaner plots, as observed. But if this is the correct explanation one would expect it to be due to competition between the weeds and the barley for the nutrients and in particular for the available nitrogen. Now the barley crop showed very marked deterioration in yield in the second three-year period, but this deterioration has been shown to be the same on the comparatively clean ploughed plots as on the dirty grubbed ones, and is six or seven times as large as the loss of yield due to using the grubber. The competition between the weeds and the barley for nutrients must, therefore, have been very small if it were the cause of lowering of yield of the grubbed compared with the ploughed plots.

The mangold crop appears to be more affected by the weediness than by the tilth of the seed-bed, though probably in each year the variations in tilth from plot to plot were considerably smaller than the variations in weediness. Weeds compete very successfully with the young mangold plants in the first stages of their growth either by killing them off or by giving them a set-back from which they never completely recover. This is the probable cause of the harvest observation that the mangold leaves go yellow soonest on the shallow non-ploughed plots and keep greenest longest on the deep ploughed plots for this is the order of seriousness of weed competition in the seed-bed. This argument is barely invalidated, as it seems to be for the barley crop, by the result that the rate of deterioration of yield is the same on the ploughed and the non-ploughed plots, for after the first 2-3 weeks of crop growth all the mangold plots are kept equally clean by hoeing for the rest of the growing season. There is only a greater shortage of nutrients on the unploughed than on the ploughed plots for the very early and not for the greater part of the growing season but, as Brenchley (1929) has shown, the future physiological development of the crop is especially sensitive to shortage just at this time.

Thus while no definite conclusions can be drawn from the experimental data on whether less suitable tilth or increased weediness is the cause of the crops on some cultivation treatments yielding less than on others, the generalization that winter wheat and mangolds are more susceptible to weeds and less to seed-bed tilth than barley appears to be consistent with the experimental results.

THE PRACTICAL CONCLUSIONS FROM THESE RESULTS

The main result that has emerged from this experiment is the very great importance of good weed control in the seed-bed. The great virtue of preparing a seed-bed by ploughing and then breaking up the furrow with harrows and rolls is that by turning the furrow slice right over, perennial and annual weeds are kept in check while the young crop is germinating and beginning to grow. Good tilth by itself does not ensure this condition, and in fact this experiment gave the impression that the development of the crop was not very sensitive to variations in the tilth of the seed-bed provided it was clean.

This result, which is in accord with British agricultural practice, means that the plough, by turning over the furrow slice can be a most effective controller of weeds in the early stages of the crop development, and no method of seed-bed preparation that does not ensure this burying of weeds and weed seeds can be of more than limited use.

But this result also means that if the land is already clean, as it often is after a root crop, any method that gets a reasonable tilth quickly can be used without the necessity for a ploughing.

The second general conclusion is that except possibly for the mangold crop the depth of working the seed-bed appeared to be of no importance, since there was no appreciable difference in yield between plots ploughed to a 4 in. or to an 8 in. depth for the whole of the six-year period, the largest difference being about 5 % for the mangold crop. This must not be taken to mean that ploughing below 4 in. is never beneficial, but it does mean that, as far as crop yield at Rothamsted is concerned, if the soil has been ploughed to below 4 in., it is probably unnecessary to repeat this depth for at least six years. Ploughing to 8 in. did, however, appear to depress the weed population rather better than ploughing to only 4 in., and this beneficial action is probably more important the more weedy the land being ploughed.

A corollary follows from these two conclusions, namely that the higher yield usually obtained by going over the land twice with either the rototiller or the grubber to get an 8 in. depth of working rather than only once to get a 4 in. depth was almost certainly due to the better weed control obtained. Hence if the land is not quite clean it is still unnecessary to plough it provided the cultivators go over the land more than once so that they thoroughly disturb all the weeds present.

SUMMARY

1. The results of a six-year cultivation rotation experiment are given. The rotation used was wheat-mangolds-barley and the seed-beds for these were prepared either by ploughing, using a rotary cultivator or a tractor-drawn grubber.

2. The yields of these crops were barely influenced by the depth of ploughing, a 4 in. depth giving throughout the six years just about the same yield as an 8 in. depth. The mangold crop was possibly a little larger on the deeper ploughed plots.

3. The mean yields of the seed-beds prepared with the tractor drawn grubber or cultivator followed by harrows etc. were lower than the ploughed seed-beds for all the crops, and this was particularly so on those seed-beds prepared by only one grubbing down to 4 in. depth.

4. The mean yields on the seed-beds prepared by the rototiller were lower than on the ploughed seed-beds for wheat and mangolds. If the seed-bed was prepared by rototillage to a depth of 8 in. by going over the land twice, the yield of barley was the same as on the ploughed seed-beds, but was definitely less on the seed-bed rototilled only once to 4 in.

5. Seed-beds prepared by the rototiller or grubber have only a small residual effect on the crop yields in the following year.

6. It is concluded that the primary function of ploughing is weed control, and that it is only advisable to omit ploughing either if the land is already fairly clean or if the crop will be hoed very early on in its development.

7. For wheat and mangolds differences in weed infestation of the seed-bed were probably of greater importance than differences in tilth in so far as the crop yield was concerned. The reverse may have been true for barley.

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GAS AND VAPOUR MOVEMENTS IN THE SOIL

I. THE DIFFUSION OF VAPOURS THROUGH POROUS SOLIDS

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(With Five Text-figures)

THE greater part of the research work on soils is, and has been, understandably devoted to the properties of the solid and liquid parts of the soil system, and a survey of the literature shows that the attention paid to the physics of the soil atmosphere is much less than its biological importance deserves. Physically, the most important problem associated with the soil atmosphere is the investigation of the mechanism of gas and vapour movements in the soil, such as the gaseous exchange of oxygen from the outside atmosphere for the carbon dioxide being produced in the neighbourhood of plant roots or by micro-organisms in the soil. Keen (1931) has provided a survey of this problem based on the work of Romell (1922) and Buckingham (1904), and shows that whilst changes in the soil temperature, changes in the barometric pressure, and the effects of wind, rain and evaporation may all assist in the process, gaseous diffusion provides the only continuously operating mechanism capable of accounting quantitatively for the observed gas movements. The numerical calculations of the rate of exchange depend on Buckingham's finding that the rate of diffusion of a gas through a porous solid is approximately proportional to the square of the porosity (fractional part of the system not occupied by solid or liquid) and is independent of the moisture content and texture of the soil. His experimental points show a large scatter and the parabolic law can be regarded as a first approximation only. In an endeavour to obtain a more precise relation, Smith & Brown (1933) repeated Buckingham's experiments but found, with their moist field soils, that reliable diffusion coefficients were unobtainable because of the evolution of carbon dioxide by micro-organisms in the test samples, and it is possible that Buckingham's results were similarly influenced. As the dependence of the rate of diffusion upon porosity is of considerable importance to the plant

physiologist a re-examination of the question needs little justification, and the results will be of interest in other problems. For instance, in using soil fumigants the spreading of the toxic vapour is primarily a diffusion process, and knowledge of the diffusion-porosity law will enable the optimum depths, spacings and quantities of injection to be forecast to achieve a required space-time distribution of vapour. Some empirical data on the spreading of carbon disulphide vapour have been obtained by Higgins & Pollard (1937), and although their experimental conditions involve complex boundary conditions in the mathematical analysis, it has been possible to make reasonable simplifying assumptions and to show that an adequate description of their results can be obtained by considering the vapour movement as diffusion.

The reduced rate of diffusion through a porous body is due in part to the reduced area of cross-section available for gas movements and in part to the increased path length imposed by the tortuous nature of the channels which the molecules must follow. We should thus expect, for a given porous solid, that the relation between the steady state rate of diffusion and porosity will be the same for all gases and vapours and may be expressible in the form $D = D_0 f(S)$, where D is the coefficient of diffusion through a material of pore-space S , and D_0 is the coefficient of diffusion through free air ($S = 1.0$). In Buckingham's equation, $f(S) = S^2$.

Acceptance of this general law enables a considerable simplification of the experimental technique to be made. Instead of using a gas such as carbon dioxide, necessitating complex arrangements for producing and maintaining a known partial pressure on one face of a soil sample, and some means, either chemical or physical, for estimating the rate of flow through the sample, a vapour such as carbon disulphide or acetone can be used, the rate of evaporation of the liquid being measured by direct weighing. The partial pressure gradient is readily obtained, because the pressure difference across the faces of the soil sample is merely the saturated vapour pressure of the liquid at the temperature of the experiment. After a preliminary experiment the dimensions of the apparatus necessary to give reasonable accuracy in all measurements which must be made are readily determined. A description of the apparatus appears below (p. 446).

The present report includes a study of the dependence of D upon S for steady state conditions and also an account of preliminary work on the effects of moisture and adsorption in the non-steady state. The effects of chemical and biological action are not considered.

THEORETICAL

Steady state conditions. If the partial pressures of a diffusing gas be maintained at values p_1 and p_2 at two parallel planes distance l apart in air, the total pressure being uniform throughout the system, there is a steady flow of gas in one direction and an equal flow of air in the opposite direction. If the area of cross-section is A the rates of flow are given by

$$\frac{dq}{dt} = \alpha A \frac{p_1 - p_2}{l},$$

where α is a constant for a given pair of gases. It is a coefficient of diffusion, in units which depend upon those of the other quantities. Measuring A and l in cm., p in mm. of mercury, q in mg. and t in seconds, we may write $\alpha = D_0/\beta$, where β is a constant given by the equation $n = p/\beta$, n being the concentration in mg./c.c. at pressure p . The steady state equation may thus be written in two forms

$$\begin{aligned} \frac{\partial q}{\partial t} &= -\frac{D_0}{\beta} A \frac{\partial p}{\partial l} \\ \text{or} \quad \frac{\partial q}{\partial t} &= -D_0 A \frac{\partial n}{\partial l}, \end{aligned} \quad (1)$$

and is true for all planes normal to the direction of flow.

Note that D_0 is the coefficient obtained when n is measured in units of q per c.c.

Non-steady state conditions. When there is not a uniform pressure gradient, equation (1) holds at all planes. Consider two planes δx apart. The amount entering the element at the first is given by

$$\frac{\partial q_1}{\partial t} = -\frac{D_0}{\beta} A \frac{\partial p}{\partial x},$$

the amount leaving at the second is

$$\frac{\partial q_2}{\partial t} = -\frac{D_0}{\beta} A \left\{ \frac{\partial p}{\partial x} + \frac{\partial}{\partial x} \left(\frac{\partial p}{\partial x} \right) \delta x \right\},$$

and the net gain in the element

$$\frac{\partial q}{\partial t} = \frac{D_0}{\beta} A \frac{\partial^2 p}{\partial x^2} \delta x.$$

If n is the concentration, we have $n = q/A\delta x$, i.e. $q/A\delta x = p/\beta$ and we obtain

$$\frac{\partial p}{\partial t} = D_0 \frac{\partial^2 p}{\partial x^2}. \quad (2)$$

The extension to a three-dimensional case yields

$$\frac{\partial p}{\partial t} = D_0 \left\{ \frac{\partial^2 p}{\partial x^2} + \frac{\partial^2 p}{\partial y^2} + \frac{\partial^2 p}{\partial z^2} \right\} = D_0 \nabla^2 p. \quad (2a)$$

Equation (2), or its more general form (2a), formally defines the coefficient of diffusion D_0 . The equation is the same as that for the flow of heat in a conducting solid if we replace p by θ , the temperature at any point, and the solution of the diffusion equation in the various practical cases which are presented will follow the same lines as the analogous problems in heat conduction, most of which are adequately dealt with in text books on Fourier analysis.

Diffusion through a porous solid. When the inter-diffusion of gas and air is restricted to movement through the pores of a solid body, the amount of material which can pass across a given plane is reduced because of the smaller area of the cross-section available, and effective pressure gradients are reduced because of the tortuous nature of the paths which the gas molecules must take. Let us define the coefficient of diffusion, D , for a solid of porosity S , in terms of the steady state transfer of mass, by the equation

$$\frac{\partial q}{\partial t} = - \frac{D}{\beta} A \frac{\partial p}{\partial x}. \quad (3)$$

For the non-steady state we obtain

$$\frac{\partial q}{\partial t} = \frac{\partial q_1}{\partial t} - \frac{\partial q_2}{\partial t} = \frac{D}{\beta} A \frac{\partial^2 p}{\partial x^2} \delta x.$$

In this case the quantity q is present in volume $A \delta x S$, i.e.

$$\frac{q}{A \delta x S} = \frac{p}{\beta}$$

and

$$\frac{\partial q}{\partial t} = \frac{A \delta x S}{\beta} \frac{\partial p}{\partial t},$$

i.e.

$$\frac{\partial p}{\partial t} = \frac{D}{S} \frac{\partial^2 p}{\partial x^2}. \quad (4)$$

From equations (3) and (4) an important distinction is established which must be borne in mind when discussing "rate of diffusion". The rate of redistribution of mass is dependent on D (equation (3)); the rate of redistribution of pressure is dependent upon D/S (equation (4)). The above derivation implicitly assumes that the porous solid is isotropic, i.e. that the pore-space is randomly distributed and that in any area of cross-section the fraction not occupied by solid will be S . This will probably be true for most granular solids whose particles approximate

to spheres, i.e. we expect equations (3) and (4) to hold for most soils and sands but anticipate that they will break down for laminated solids such as mica. If the total area of cross-section of the solid is A , the effective area across which diffusion takes place is SA . The effective path length through the solid will be greater than l : let it be l_e . Then in the steady state we may consider the diffusion through a solid of length l and cross-section A as being the same as that through a column of air of length l_e and area SA , i.e. we have

$$\begin{aligned}\frac{dq}{dt} &= \frac{D}{\beta} A \frac{p_1 - p_2}{l} \\ &= \frac{D_0}{\beta} AS \frac{p_1 - p_2}{l_e} \\ \text{or} \quad D &= D_0 S \frac{l}{l_e}.\end{aligned}\tag{5}$$

Recent work on the viscous flow of liquids through soils (Carman, 1939) indicates that over a wide range of values of S the liquid moves as though its actual path through the soil made an angle of 45° with the direction of the maximum pressure gradient, i.e. l/l_e is approximately $1/\sqrt{2}$. As the diffusing gas molecules must follow the same paths we may anticipate the establishment of the following relation, for a certain range of S at least,

$$D = D_0 S / \sqrt{2}.$$

General treatment of diffusion measurements. For the steady state we have

$$\frac{dq}{dt} = \frac{D}{\beta} A \frac{p_1 - p_2}{l}.$$

The value of D depends upon the absolute temperature and the total pressure, the relation for most gases and vapours being

$$D_{T,P} = D \left(\frac{T}{273} \right)^2 \frac{P_0}{P},$$

where P_0 is 1 atm. The constant β is a function of temperature, since $1/\beta$ is the concentration in mg./c.c. at 1 mm. pressure, and we have

$$\beta = \beta_0 \frac{T}{273}.$$

Thus we have for the rate of flow in mg./sec.

$$\begin{aligned}\frac{dq}{dt} &= DA \frac{p_1 - p_2}{l} \left(\frac{T}{273} \right)^2 \frac{P_0}{P} \beta_0 \left(\frac{T}{273} \right) \\ &= \left(\frac{p_1 - p_2}{\beta_0} \frac{T}{273} \right) \left(D \frac{A P_0}{l P} \right),\end{aligned}$$

which we may write

$$C = E \times 1/Z$$

or $\text{current} = \text{diffusion potential difference} \div \text{impedance}$. As indicated above, C will be measured directly, E is a function of temperature only and a table of values can be drawn up for ready reference.

A typical value of E is obtained as follows. At 15°C . the vapour pressure of carbon disulphide is 242 mm. The value of β_0 is $\frac{760 \times 21,900}{76 \times 1000} = 219$, since 76 g. CS_2 vapour occupy 21,900 c.c. at 0°C . and 760 mm. pressure. Therefore

$$E_{15.0} = \frac{242}{219} \times \frac{288}{273} = 1.165.$$

When E and C are known, Z can be calculated and corrected to 1 atm. A and l are constants of the apparatus and hence D can be found.

Solution of non-steady state equation. As already indicated above, solutions of the general equation for various boundary conditions can be obtained from standard mathematical texts. The results of the standard method of treatment for the special conditions which will be considered in the experimental section below are therefore given without detailed working. Consider a cylinder, length l , area of cross-section A , closed at one end and filled to the top with a porous solid such as sand or a soil. Imagine a small amount of liquid injected at a depth h below the open surface such that it forms a thin sheet of negligible thickness across the cylinder. The vapour will diffuse upward and downward, and we assume that any vapour passing out of the open surface is immediately carried away so that there is no accumulation of vapour in the atmosphere just above the cylinder. The emission of vapour from the surface can be considered in three stages: (a) there is a period during which the rate of emission increases from zero as the vapour begins to diffuse from depth h to the surface, (b) there is a steady state which begins when a uniform pressure gradient has been set up between h and the surface, and this lasts until all the liquid has evaporated, and (c) there is a final "decay" state following on (b), the pore-space between h and l being full of vapour at the beginning with a uniform gradient between h and 0. Stage (c) ends when all the vapour has passed out of the system.

Stage a ("growth" stage). The equation to be satisfied is

$$\frac{\partial p}{\partial t} = \frac{D}{S} \frac{\partial^2 p}{\partial x^2} \quad \text{for } t > 0.$$

The boundary conditions are

- (i) At $t=0$, $p=0$ for all values of x except $x=h$.

(ii) For all t , $p = p_0$ at $x = h$ (p_0 = vapour pressure of liquid),

$p = 0$ at $x = 0$ (i.e. at surface),

$\frac{\partial p}{\partial x} = 0$ at $x = l$ (i.e. no flow across the bottom of the cylinder).

The solution is in two parts:

In the range $h > x > 0$

$$p = p_0 \frac{x}{h} + \frac{2p_0}{\pi} \sum_{n=1}^{\infty} (-1)^n \frac{1}{n} \exp \left[-\frac{n^2 \pi^2 D}{h^2 S} t \right] \sin \frac{n\pi x}{h}.$$

In the range $l > x > h$

$$p = p_0 - \frac{4p_0}{\pi} \sum_{n=0}^{\infty} \frac{1}{2n+1} \exp \left[-\left(\frac{2n+1}{l-h} \frac{\pi}{2} \right)^2 \frac{D}{S} t \right] \sin \frac{2n+1}{2} \frac{\pi}{l-h} (x-h),$$

a result which is only needed to provide analytical confirmation of the eventual uniformity of pressure ($p = p_0$ for all values of x) for large values of t .

The rate of emission from the open end depends upon the pressure gradient at $x = 0$. We have,

$$\frac{\partial p}{\partial x} = p_0 \frac{1}{h} + \frac{2p_0}{\pi} \sum_{n=1}^{\infty} (-1)^n \frac{1}{n} \exp \left[-\frac{n^2 \pi^2 D}{h^2 S} t \right] \frac{n\pi}{h} \cos \frac{n\pi x}{h}$$

and at $x = 0$ this becomes

$$\frac{\partial p}{\partial x} = p_0 \frac{1}{h} + \frac{2p_0}{h} \sum_{n=1}^{\infty} (-1)^n \exp \left[-\frac{n^2 \pi^2 D}{h^2 S} t \right].$$

From equation 3 we have, remembering that $\partial p / \partial x$ is positive in this case,

$$\begin{aligned} \frac{\partial q}{\partial t} &= \beta A \frac{\partial p}{\partial x} \\ &= \beta A \frac{p_0}{h} \left\{ 1 + 2 \sum_{n=1}^{\infty} (-1)^n \exp \left[-\frac{n^2 \pi^2 D t}{h^2 S} \right] \right\}. \end{aligned} \quad (6a)$$

As $t \rightarrow \infty$, this becomes

$$\frac{\partial q}{\partial t} = \beta A \frac{p_0}{h}, \quad (6b)$$

which is the equation for the steady state (b) in which the pressure gradient is uniform and equal to p_0/h . The series generally converges rapidly and in practice few terms are necessary even for small values of t .

Stage c ("decay" stage). With a new zero for time, i.e. measuring time from the instant at which the last part of the liquid vaporizes, we have the following conditions at $t = 0$. There is a uniform vapour pressure

(p_0) between $x=l$ and $x=h$ and a uniform pressure gradient (p_0/h) between $x=h$ and $x=0$. We have, as before,

$$\frac{\partial p}{\partial t} = \frac{D}{S} \frac{\partial^2 p}{\partial x^2} \quad \text{for } t > 0.$$

Also,

$$\left. \begin{aligned} p &= p_0 & \text{for } l > x > h \\ p &= p_0 \frac{x}{h} & \text{for } h > x > 0 \end{aligned} \right\} \text{ at } t=0,$$

$$\frac{\partial p}{\partial x} = 0 \quad \text{at } x=l \quad \text{for all } t,$$

$$p = 0 \quad \text{at } x=0 \quad \text{for all } t,$$

and the solution is

$$p = \frac{8p_0 l}{h} \sum_0^{\infty} \frac{1}{(2n+1)^2 \pi^2} \exp \left[- \left(\frac{2n+1}{2l} \pi \right)^2 \frac{D}{S} t \right] \sin \frac{2n+1}{2l} \pi h \sin \frac{2n+1}{2l} \pi x,$$

$$\frac{\partial p}{\partial x} = \frac{8p_0 l}{h} \sum_0^{\infty} \frac{1}{(2n+1) \pi \cdot 2l} \exp \left[- \left(\frac{2n+1}{2l} \pi \right)^2 \frac{D}{S} t \right] \sin \frac{2n+1}{2l} \pi h \cos \frac{2n+1}{2l} \pi x,$$

and at $x=0$

$$\frac{\partial p}{\partial x} = \frac{4p_0}{h} \sum_0^{\infty} \frac{1}{(2n+1) \pi} \exp \left[- \left(\frac{2n+1}{2l} \pi \right)^2 \frac{D}{S} t \right] \sin \frac{2n+1}{2l} \pi h,$$

and

$$\frac{\partial q}{\partial t} = \frac{D}{\beta} A \frac{p_0}{h} \frac{4}{\pi} \sum_0^{\infty} \frac{1}{2n+1} \exp \left[- \left(\frac{2n+1}{2l} \pi \right)^2 \frac{D}{S} t \right] \sin \frac{2n+1}{2l} \pi h. \quad (6c)$$

Two cases will be considered later.

(i) Put $h=l$, i.e. the diffusion takes place from the bottom of the cylinder. The values for the three stages become

$$(a) \quad \frac{\partial q}{\partial t} = \frac{D}{\beta} A \frac{p_0}{l} \left\{ 1 + 2 \sum_1^{\infty} (-1)^n \exp \left[- \frac{n^2 \pi^2 D t}{l^2 S} \right] \right\}, \quad (7 \text{ i } a)$$

$$(b) \quad \frac{\partial q}{\partial t} = \frac{D}{\beta} A \frac{p_0}{l}, \quad (7 \text{ i } b)$$

$$(c) \quad \frac{\partial q}{\partial t} = \frac{D}{\beta} A \frac{p_0}{l} \left\{ \frac{4}{\pi} \sum_0^{\infty} \frac{1}{2n+1} (-1)^n \exp \left[- \left(\frac{2n+1}{2l} \pi \right)^2 \frac{D}{S} t \right] \right\}. \quad (7 \text{ i } c)$$

(ii) Put $h=l/2$, i.e. the diffusion takes place from a plane half-way down the cylinder:

$$(a) \quad \frac{\partial q}{\partial t} = \frac{D}{\beta} A \frac{2p_0}{l} \left\{ 1 + 2 \sum_1^{\infty} (-1)^n \exp \left[- \frac{4\pi^2 n^2 D t}{l^2 S} \right] \right\}, \quad (7 \text{ ii } a)$$

$$(b) \quad \frac{\partial q}{\partial t} = \frac{D}{\beta} A \frac{2p_0}{l}, \quad (7 \text{ ii } b)$$

$$(c) \quad \frac{\partial q}{\partial t} = \frac{D}{\beta} A \frac{2p_0}{l} \left\{ \frac{4}{\pi} \sum_{n=0}^{\infty} \frac{1}{2n+1} \exp \left[- \left(\frac{2n+1}{2l} \pi \right)^2 \frac{Dt}{S} \right] \sin (2n+1) \frac{\pi}{4} \right\}. \quad (7 \text{ ii } c)$$

The presence of D/S in the exponential index of equations (a) and (c) shows that the rate of attainment of equilibrium depends upon this ratio, whilst the steady state rate depends upon D only (equation (b)).

The effects of solution and adsorption. When moisture is present in the soil it occupies part of the space which would otherwise be available for gas and vapour, and it will affect D by modifying the pore-space. There is a further effect if the diffusing material is soluble in water, as carbon dioxide and carbon disulphide are. Part of the material is removed from the vapour phase, and we may assume that the equilibrium between vapour and solution phases is instantaneously attained and that the concentration of the solution is proportional to the vapour pressure. Adsorption of the gas by the solid will also remove some of the material from the vapour phase, and if we may assume that the concentration in the condensed phases is proportional to the concentration in the vapour phase, we can treat both effects together. As in the few cases so far examined the amount adsorbed appears to be much greater than the amount dissolved, we use the term adsorption to cover both effects.

Consider the material present at a given part of a porous system as being divided between a vapour phase, exerting a pressure p , and an adsorbed phase in equilibrium with it. Let N mg./c.c. of system be the total concentration, and let n mg./c.c. of system be the vapour concentration. Then $p = \beta n/S$. We assume that $n/N - n$ is independent of N , i.e. n/N is constant, and $=\gamma$ say. The steady state will not be affected; once a uniform pressure gradient is set up our previous equation will hold:

$$\frac{\partial q}{\partial t} = - \frac{D}{\beta} A \frac{\partial p}{\partial x}. \quad (3)$$

For the non-steady state, the equation

$$\frac{\partial q}{\partial t} = \frac{D}{\beta} A \delta x \frac{\partial^2 p}{\partial x^2}$$

is modified by setting

$$q = A \delta x N = A \delta x \frac{n}{\gamma} = A \delta x \frac{pS}{\beta\gamma},$$

i.e.
$$\frac{\partial q}{\partial t} = \frac{A \delta x S}{\beta \gamma} \frac{\partial p}{\partial t},$$

or
$$\frac{\partial p}{\partial t} = \frac{\gamma D}{S} \frac{\partial^2 p}{\partial x^2}. \quad (8)$$

The coefficient of pressure diffusion, previously D/S , thus becomes $\gamma D/S$, and as γ is less than unity the effect of adsorption is to reduce the rate at which pressure redistribution takes place, and the non-steady state rates of emission from the cylinder (i) (a) and (c), (ii) (a) and (c), given above, will be modified by the inclusion of a further factor γ in the exponential index. For example equation (7 i c) becomes

$$\frac{\partial q}{\partial t} = \frac{D}{\beta} A \frac{p_0}{l} \left\{ \frac{4}{\pi} \sum_{n=0}^{\infty} \frac{1}{2n+1} (-1)^n \exp \left[- \left(\frac{2n+1}{2l} \pi \right)^2 \frac{\gamma D t}{S} \right] \right\}, \quad (9 \text{ i c})$$

and corresponding equations (9 i a), (9 ii a) and (9 ii c) can be written down.

EXPERIMENTAL

Apparatus. The cylindrical brass diffusion apparatus used for vapours is shown in section in Fig. 1. A collar, *C*, screws into a reservoir *R*, and resting in a groove in *C* is a stiff disk of copper gauze, *G*, on a rubber

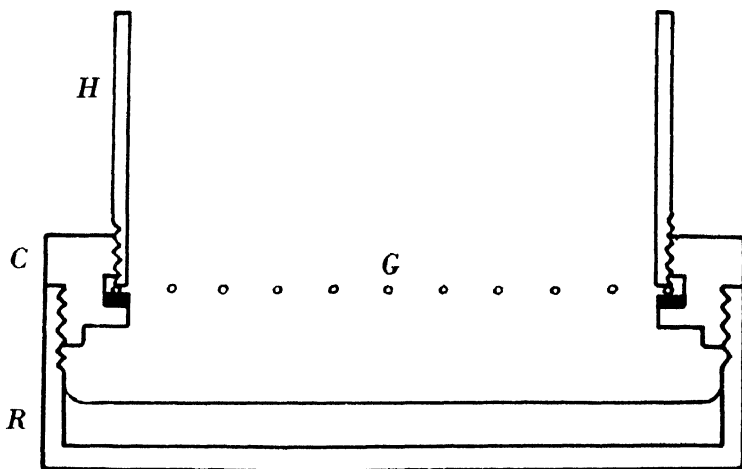


Fig. 1. Section of diffusion apparatus.

gasket. Screwed down hard on *G* is a short length of brass tubing, *H*, forming the soil holder. In practice, *C* and *H* form one unit—referred to below as the holder—and the reservoir a second unit. The original intention was to have *G* replaceable so that different size meshes could

be used, but as fine gauze cannot be kept flat, especially with a load on it, a permanent stiff grid is now maintained at G , and this is used as a support for finer gauzes necessary to prevent the solids from slipping through the grid. The dimensions are

H depth = 2.64 cm.; area = 22.3 sq. cm.

R depth = 1.6 cm.; area = 34 sq. cm.

Total weight, empty = 235 g.

Total weight, full, varies from 248 to 360 g.

Preliminary experiments with carbon disulphide showed that a suitable balance should weigh to 10 mg. and be capable of taking loads up to 400 g. The balance employed is an Avery balance, weighing up to 500 g., fitted with a pointer and scale reading from 0.00 to 1.00 g. and graduated in $\frac{1}{100}$ g. The fitting of a lens enables readings to half a division to be made, i.e. to 5 mg.

The method of experiment is to pour a known volume of liquid into R , screw in the soil holder and contents, counterpoise on the balance and then determine the rate of loss of weight by reading the pointer at intervals. The balance is in a large case with doors at the back and front, and by keeping both slightly open there is sufficient ventilation to ensure that the vapour pressure above the soil never reaches a value large enough to invalidate the assumption that it is maintained at zero. Too violent a through draught sets the balance pans swaying, and errors arise from the consequent disturbance of the liquid level in the reservoir. A thermometer hangs in the balance case and the temperature is recorded as part of every observation. Readings are usually taken every 20 min., and an experiment lasts about 360 min. The method of determining D from the results will be illustrated later after the reservoir correction has been considered.

Liquids used. Carbon disulphide has been the principal material used, as its vapour pressure at ordinary temperatures is sufficient to produce measurable rates of evaporation even through solids of very low porosity, but not so great as to have large temperature variations. It has the further advantage that it is a commercial soil fumigant. Acetone is not quite so good as an experimental liquid, and fewer results have been obtained with it.

Porous solids. A wide range of granular solids has been used so that all sorts of shapes and sizes of pores might be available. A wide range of pore-space values has been covered, and sufficient soil experiments have been included to show that soils conform to the general behaviour.

Values of D_0 for carbon disulphide and acetone. The values quoted in the literature for the coefficient of diffusion of carbon disulphide into air vary from 0.088 (*I.C.T.*) to 0.0995 (Mellor, 1925*a*). As the value of D_0 is required in later calculations a redetermination was made. The rate of evaporation of carbon disulphide from the bottom of a cylindrical jar was measured, and, assuming a uniform partial pressure gradient when the steady state was attained, an estimate of D_0 was made. The mean of five determinations gave $D_0 = 0.103 \text{ cm.}^2/\text{sec.}$

This value is a few per cent higher than any previously recorded, and the difference can be accounted for by our use of an approximation. The basic theory outlined in the preceding pages only holds for two gases interdiffusing because of small partial pressure gradients; the experiments were concerned with one-way motion of a vapour through a stationary gas (air), and the partial pressure gradients were large. The effect of the latter is to introduce an error of 1 or 2 % due to the variation of the coefficient of diffusion with the relative proportions of the vapour-air mixture, whilst the effect of the one-way motion is to produce a non-linear partial pressure gradient in the steady state, leading to a further error, in the same sense, of a few per cent. The value of D_0 is, therefore, some 5–10 % larger than the true coefficient of diffusion of carbon disulphide vapour into air, but as the nature of the correction will be the same for all the determinations of D reported below, the ratio D/D_0 will be free from significant error. There will, of course, be an uncertainty of 2 or 3 % in the values of the ratio, but we shall see that this is less than the scatter of the experimental results for porosities of technical interest, and the order of accuracy obtained will be quite adequate for the present purpose. Until there is a need for more precise measurements, one may reasonably ignore second-order corrections, whether arising from this approximation to the basic theory, the uncertainties of which have been outlined by Chapman (1928), or from the criticisms of the evaporation method of measuring diffusion coefficients which have been made by Trautz & Müller (1935). In a separate account a more exhaustive discussion of the physical aspects of the problem will be provided; further elaboration is unnecessary here.

Similar determinations were made for acetone, the mean of two giving $D_0 = 0.095 \text{ cm.}^2/\text{sec.}$ There does not appear to be any record of a previous determination of the coefficient of diffusion of acetone vapour into air.

Reservoir correction. As will be seen from Fig. 1, the vapour-pressure drop between the surface of the liquid and the surface of the soil is not

effectively applied to the faces of the soil. There is a drop between the liquid surface and the bottom of the soil sample, and a further drop across the sample, and it is this latter which we need for calculations of diffusion rates. The necessary reservoir correction is obtained as follows.

Let Z_0 be the impedance of the reservoir and gauze together and let Z be the impedance of the material in the holder. The total impedance is thus $Z_0 + Z$, and if the diffusion potential difference is E , the observed diffusion current, C , will be given by $Z + Z_0 = E/C$. If now an experiment be performed with air in the holder, we have $Z = l/D_0 A$, and hence Z_0 can be calculated. The value of Z_0 is found to be practically independent of the amount of liquid in the reservoir, indicating that the main part of it is due to the impedance of the gauze. The values of Z_0 obtained with (a) a disk of 200-mesh copper gauze on G , (b) a disk of silk fabric on G , are

(a) $Z_0 = 0.76$, (b) $Z_0 = 0.78$ for CS_2 , (b) $Z_0 = 0.88$ for acetone.

Steady state determination of variation of D with S . The routine followed is to pack the granular solid into the holder, without undue pressure so that G is not strained. The holder and sample are weighed, and the weight of the holder and density of the solid being known, the volume of the solid is found. This is expressed as a fraction of the volume of the cylinder H . The liquid is poured into the reservoir, the two parts screwed together, and a rubber band slipped over the line of contact of the two parts to ensure that there is no leakage from the reservoir. Observations of time, weight and temperature are made at half-hour intervals and the mean diffusion current (c) found. The calculation then proceeds as follows.

Rothamsted subsoil (air-dry) on fabric; 8 c.c. CS_2 . Barometer 29.30 in.

Weight of soil + holder = 222.25 g.

Weight of holder = 151.47 g.

Weight of soil = 70.78 g.

Volume of soil = $\frac{70.78}{2.50} = 28.3$ c.c.

Volume of holder = 58.8 c.c. $\therefore S = 0.518$.

Experiment lasted 360 min. Readings during the first 80 min. were ignored.

$\bar{C} = 2.260 \times 10^{-1}$ mg./sec. $\theta = 12.7^\circ \text{C}$.

$E_{12.7} = 1.058$

$\therefore Z + Z_0 = 4.54$ $l = 2.64$ cm.

$(Z + Z_0)_{30} = 4.64$ $A = 22.30$ cm.²

$Z = 3.86$.

$\therefore D = \frac{2.64}{22.30} \times \frac{1}{3.86} = 0.0307 = 0.103 \times 0.298$.

A complete list of results obtained in this way appears in Table I, and a graphical representation in Fig. 2. The results for carbon disulphide and acetone through air-dry solids, and for carbon disulphide through moist soils are all included in Fig. 2 by taking D/D_0 as ordinate. The steady state results for moist soils involve a correction for the moisture which evaporates during an experiment, and it is assumed that the water-vapour loss proceeds at a uniform rate. An estimate of its amount is obtained by weighing soil and holder when all the carbon disulphide has passed out, i.e. when the rate of loss becomes constant again, and comparing with the corresponding reading taken at the beginning of the experiment. The order of magnitude of the correction will be seen

Table I

(a) Diffusion of carbon disulphide vapour

Material	S	Mean	D/D_0
		temperature ° C.	
Sand	0.357	16.6	0.249
	0.372	15.4	0.245
	0.374	15.4	0.248
	0.378	15.5	0.252
	0.381	15.6	0.252
Sand mixture	0.155	13.0	0.109
	0.164	15.6	0.124
	0.205	14.0	0.120
	0.232	15.0	0.145
	0.267	14.4	0.176
	0.275	10.8	0.168
	0.300	16.4	0.206
Common salt	0.452	14.4	0.279
	0.475	18.5	0.294
	0.545	14.4	0.350
	0.610	17.5	0.420
Talc	0.705	17.2	0.536
	0.742	14.0	0.548
	0.756	15.3	0.590
Kaolin	0.772	16.0	0.598
	0.782	17.6	0.600
Kieselguhr	0.844	15.6	0.677
	0.924	15.0	0.805
Steel wool	0.93	16.9	0.815
Glass spheres:			
Large, $d = 3$ mm.	0.397	16.7	0.319
Small, $d = \frac{1}{2}$ mm.	0.364	17.0	0.282
Mixture	0.185	15.5	0.151
Mica	0.85	14.2	0.494
	0.88	18.8	0.304
	0.89	18.2	0.380

Table I (*continued*)

(ii) Soils				
Material	Moisture content %	S	Mean temperature °C.	D/D_0
Rothamsted subsoil	Air-dry	0.518	12.7	0.298
	"	0.518	13.8	0.304
	"	0.547	14.3	0.346
	"	0.550	16.5	0.358
	11.0	0.438	18.4	0.278
	17.0	0.448	16.7	0.297
	25.0	0.626	17.2	0.442
	29.6	0.549	16.8	0.364
Rothamsted subsoil + sand	Air-dry	0.422	15.1	0.273
Natal soil (N 64)	"	0.496	16.8	0.300
	"	0.620	16.4	0.417
	"	0.676	17.1	0.475
	11.0	0.475	17.0	0.312
	19.3	0.355	19.4	0.249
	22.0	0.425	14.9	0.299
	30.6	0.195	17.0	0.118

(b) Diffusion of acetone vapour

Material	S	Mean temperature °C.	D/D_0
Mixture	0.155	14.4	0.105
Sand	0.355	17.0	0.244
Rothamsted subsoil	0.537	16.3	0.307
N 64	0.622	16.2	0.410
Kaolin	0.772	14.7	0.566
Kieselguhr	0.844	14.4	0.677
"	0.924	13.7	0.792

from some data given below (p. 455) or from Fig. 3, where the "mean zero line" represents the steady water loss.

Six points have been specially marked on Fig. 2. Three represent results for diffusion through systems of glass spheres (Δ); in descending order of D/D_0 , large, small, and a mixture of large and small spheres. The other three points (\square) represent results for mica with different types of packing. The largest value of D/D_0 was obtained when the holder was packed with its cylindrical axis horizontal. For reasons detailed below these six points have been ignored in drawing the curve.

DISCUSSION OF RESULTS FOR STEADY STATE

With the exceptions noted, the experimental points lie on or near a curve which passes through the origin. Before discussion the implications of this curve, some consideration must be given to possible sources of experimental error.

Temperature changes. Variations in temperature in the course of an experiment will cause expansion or contraction of the air-vapour mixture, thus disturbing the assumed steady state. In practice the total change was rarely more than $1^{\circ}\text{C}.$, and being spread over several hours the possible effects can be neglected.

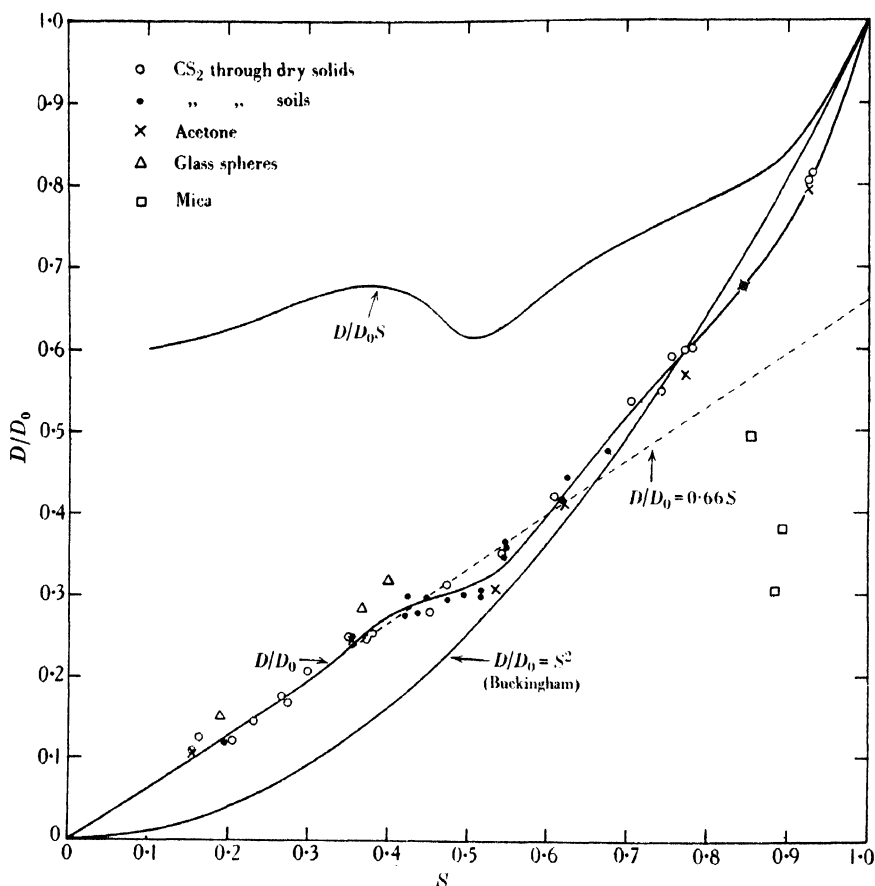


Fig. 2. Dependence of coefficient of diffusion on porosity.

Measurement of time and weight. These are two measurements which can usually be taken as absolute. The balance scale was periodically recalibrated, and as in the aggregate a determination of D depends on the measurement of a change in weight of several grammes, the readings are probably correct to 1 part in 500.

Measurement of pore-space. This depends on correct determinations of the density of the solid and of the volume of the holder. The latter

is a constant of the experiments, and any error in its determination will cause a displacement of all points but will not affect the scatter of the points. The sensitivity of S to errors in the density determinations varies, being given as $\delta S = (1 - S) \delta \rho / \rho$. Thus, assuming an error of 1% in ρ , the errors in S will be: 0.001 at $S = 0.90$, 0.005 at $S = 0.50$, and 0.009 at $S = 0.10$. Most of the density measurements were more accurate than this, particularly between $S = 0.1$ and $S = 0.4$, where the materials employed were chiefly sands or mixtures of sands.

Packing "errors". The theoretical discussion above (p. 440) is based on the assumption that the solids are randomly packed and that there is no marked anisotropy in any of the systems. This is an ideal state of affairs and in practice we cannot expect anything better than a good approximation to this ideal state. There are three sources of packing errors: (a) With large particles in the holder, edge effects are to be anticipated, because there will be comparatively straight paths available for vapour molecules to pass along the walls of the holder. This leads to an over-estimate of the coefficient of diffusion, and as this condition is considered to have arisen in the experiment with large glass spheres, the corresponding point on the graph has not been given any weight in drawing the curve. (b) Small particles may fill the meshes of the supporting gauze and thus increase the impedance of the lower system. Errors arising in this way cannot be entirely eliminated, but they have been cut down as far as possible by choosing the more suitable of the two supports employed, i.e. either the 200-mesh gauze or the silk fabric, according to the particle size of the experimental material. (c) The formation of air pockets and culs-de-sac in packing, particularly with mixed sizes of particles, will affect diffusion rates, and it is thought that this is the major cause of the scatter of the experimental points. The marked effect of anisotropy of structure is shown very clearly in the results for mica, and it is obvious that general conclusions from the data will not apply to this or any other laminated solid.

Atmospheric changes. Throughout this discussion it has been assumed that the air into which diffusion takes place is of fixed composition. This is not so, and a small part of the scatter can reasonably be attributed to variations in relative humidity.

Both of these last-mentioned sources of scatter will be present in field experiments, and we may therefore accept the mean curve as showing how an ideal field soil would affect diffusion of gases and vapours through it.

The experimental results plotted in Fig. 2 show several points of

interest. In the first place, the curve drawn is adequate for both carbon disulphide and acetone vapours, a result anticipated in the preliminary survey. Comparing the curve with the parabolic curve obtained by Buckingham for carbon dioxide, we find a marked divergence for values of S below 0.7, and this divergence is greatest in the region of practical importance, namely for values of S below 0.50. *A priori* one would expect carbon dioxide to show the same behaviour as the vapours, and a probable explanation of the conflicting experimental evidence will be given in discussing the effects of adsorption. For the moment, we accept the new curve as universally true, and note that diffusion is even less affected by the solid than Buckingham found it to be. The scatter of the experimental points introduces some uncertainty into the drawing of the best curve, and it is possible that the decrease in slope between $S=0.40$ and $S=0.55$ is not as pronounced as the curve suggests. The decrease is shown more strikingly in the plotting of D/D_0S against S . (This has been plotted from the curve and not from the experimental points.) The derived function represents the ratio l/l_e (equation (5)) which will be dependent on the pore-geometry of the solid, but before any conclusions about the nature of the packing can be safely drawn, more detailed investigation in this porosity range will be necessary. At small porosities the derived curve is too sensitive to variations in D/D_0 to permit a useful direct comparison with Carman's results for liquid flow, and a more effective comparison can be made from the mean straight line (dotted) which has been drawn to show the dependence of D/D_0 on S in the range $0.0 < S < 0.6$, a range which covers the porosities of technical interest. The slope of this line is 0.66 and this is a measure of the ratio of the actual length of the soil column to the effective length. Carman's value, which his experimental results indicate as probably being too big, is $1/\sqrt{2}$, i.e. 0.707. The agreement is good and provides support of the assumption that diffusion through a porous body is primarily a function of the geometry of the body and is independent of the nature of the diffusing material. The effective path lengths are determined by the distances the vapour molecules must travel to pass around the solid particles, and one would expect them to be slightly shorter for spherical particles than for particles of less regular shape. The coefficient of diffusion should therefore be slightly greater through a system of spheres than through any other system of particles, and this is experimentally confirmed by the recorded values. The corresponding points lie above the curve, the departure being greatest for the large spheres, but, as we have seen, part of this can be ascribed to an edge

effect. As the primary object of this work is to obtain a curve which can be applied to soils, the results for spheres have not been considered in drawing the curve.

THE EFFECTS OF SOLUTION AND ADSORPTION

A few non-steady state experiments have been carried out with moist soils using carbon disulphide as the diffusing vapour. From the published data on the solubility of carbon disulphide in water (Mellor, 1925*b*) it is possible to make an estimate of γ (see p. 445) assuming that there is no adsorption taking place, and a theoretical "decay" curve can be drawn. The experimental curves were found to have a slower rate of decay, indicating that γ was much less than the estimate based on the solvent action of the soil moisture. The disagreement was attributed to the occurrence of adsorption and this was confirmed by weighing the soil and holder during the steady state. After making allowance for the water loss, marked increases in weight were found; these were presumably due to the adsorbed carbon disulphide. Knowing the pore-space, it was possible to calculate the weight of vapour present in the soil and thus to obtain an effective value of γ .

The design of the apparatus does not conform to that assumed in the theoretical analysis of p. 442, because of the reservoir below the soil. This is not allowed for in calculating γ , which will be somewhat underestimated. At the same time the content of the soil during the steady state has been estimated by assuming that the whole of the vapour pressure difference is exerted across the soil, leading to an over-estimate of γ . The two effects thus tend to annul each other and we may expect some sort of agreement between theory and practice if we assume that our diffusion apparatus does behave like an ideal cylinder: exact agreement will be fortuitous. Reasonable agreement has been found and a typical experiment will be quoted in detail.

Moist Rothamsted subsoil: Moisture content = 17.0%.

Barometer reading 29.80 in.

Weight of soil + holder ($t=0$ min.) = 223.81
 ($t=262$ min.) = 223.73
 ($t=500$ min.) = 222.86.

Net loss of water in 500 min. = 0.95 g.

Net loss of water in 262 min. = 0.50 g.

Actual loss in weight in 262 min. = 0.08 g.

\therefore net gain in CS₂ = 0.42 g.

$$\text{Volume of vapour} = 58.8 \times S = 26.3 \text{ c.c. } (S=0.448).$$

$$\text{Mean pressure of vapour} = \frac{1}{2} \times 261 = 130 \text{ mm. (at } 17^\circ \text{ C.)}.$$

$$\beta \text{ at } 17^\circ \text{ C.} = 219 \times \frac{290}{273} = 232.$$

$$\begin{aligned} \therefore \text{weight of vapour} &= \frac{26.3}{232} \times \frac{130}{760} \times 76 \times 10^3 \text{ mg.} \\ &= 14.7 \text{ mg.} \end{aligned}$$

$$\text{Total content of soil at 262 min.} = 420 \text{ mg.}$$

$$\therefore \gamma = \frac{14.7}{420} = 0.035.$$

Considering the decay state only, the theoretical expression (p. 446) is

$$\frac{\partial q}{\partial t} = \frac{D}{\beta} A \frac{p_0}{l} \left\{ \frac{4}{\pi} \sum_{n=0}^{\infty} \frac{1}{2n+1} (-1)^n \exp \left[- \left(\frac{2n+1}{2l} \pi \right)^2 \frac{\gamma D t}{S} \right] \right\}, \quad (9ic)$$

in which the first group of terms represents the steady state rate of emission. The exponential index contains a factor D/l^2S to which some correction for the reservoir must be applied. We have $Z=l/DA$ and if V is the volume of the system ($=lA$), $Z=l^2/DV$, i.e. $D/l^2=1/VZ$. It is proposed, as a first approximation, that instead of D/l^2S the value of $1/\Sigma VZS$ be used in the exponential index. In this particular case

$$\text{for } H \text{ we have } V=58.8, S=0.448, Z=4.11, VZS=108,$$

$$\text{for } R \text{ we have } V=41.0, S=1.00, Z=0.76, VZS=31.$$

The value of $1/\Sigma VZS$ obtained from these quantities will be that for 0° C. and 30 in., and to obtain the values for the conditions of the experiment the temperature and pressure corrections must be applied: we obtain

$$\frac{D}{l^2S} = 0.0082, \quad \therefore \frac{\pi^2 \gamma D}{4l^2S} = 7.05 \times 10^{-4}.$$

The equation of the decay state may thus be written as

$$C = C_0 \frac{4}{\pi} \sum_{n=0}^{\infty} \frac{1}{2n+1} (-1)^n \exp [-(2n+1)^2 7.05 \times 10^{-4} t].$$

After about 20 min. ($t=1200$) only one term in the series is necessary, and the equation reduces to the simple form of

$$C = C_0 \frac{4}{\pi} \exp [-7.05 \times 10^{-4} t].$$

The experimental points and theoretical curve are shown in Fig. 3. This rapid convergence of the series makes a comparison of theoretical and experimental curves somewhat simpler. If the experimental points are plotted logarithmically the decay curve becomes a straight line from the

slope of which γ can be calculated and compared with the value obtained by direct weighing of the soil and holder during the steady state. The results of two experiments on Natal soil have been treated in this way and the agreement is very good.

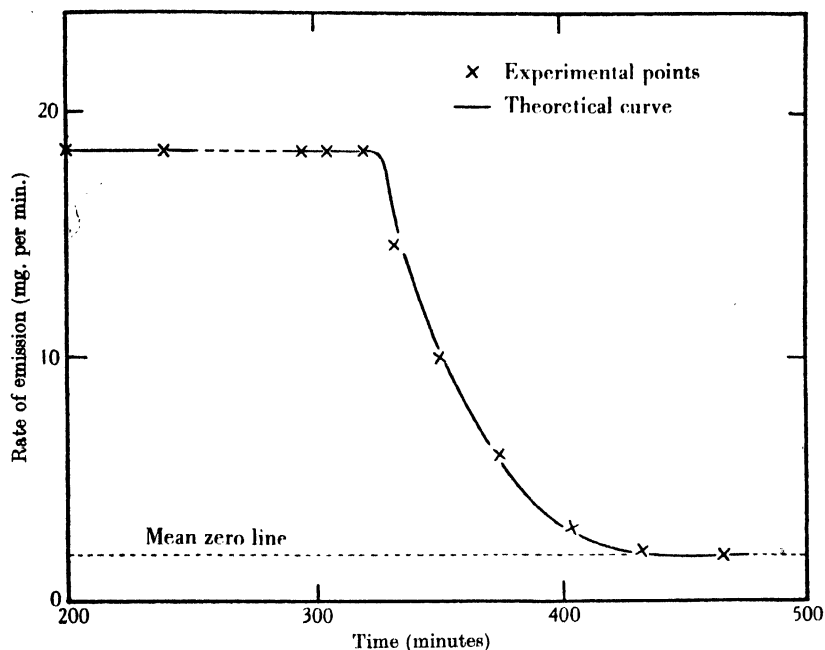


Fig. 3. Rate of emission of carbon disulphide vapour from moist Rothamsted soil.

Table II

Soil	Moisture %	S	γ (from weighing)	γ (from slope)
Natal 64	19.3	0.355	0.025	0.026
	22.0	0.425	0.026	0.024

As the theoretical basis of these experiments on adsorption has yet to be established and the apparatus used does not conform to the ideal laid down in the theoretical analysis, no useful purpose will be served by extending the above table. Taken in conjunction with Fig. 3 it demonstrates the adequacy of the technique employed in handling adsorption data, suggests that the basic assumptions are probably reliable, and indicates that with better choice of experimental conditions the theoretical prediction of the effect of adsorption will be realized in practice.

Experimental data for conditions approximating much more closely to the ideal have been provided by Dr J. C. Higgins of Imperial College, London.¹ The rate of emission of carbon disulphide vapour from the surface of soil packed in a cylinder was measured at intervals by a chemical method, two sets of experiments being performed on each soil. These correspond to the two theoretical cases examined above (p. 444), known volumes of liquid being injected (i) at the bottom, or (ii) mid-way up the cylinder. There is no direct measurement available for the extent of the adsorption, but an indirect estimate should be possible. We have assumed that the decay state begins when the last drop of liquid vaporizes and the carbon disulphide content of the soil will then consist of adsorbed material and vapour. The vapour content is easily found when the pore-space is known, and the adsorbed liquid plus vapour will be given by the amount of vapour which passes out during the decay state, and this can be determined by measuring the area of the curve between the time of onset of decay and infinity. The scatter of the experimental points makes it difficult to decide when the steady state ends, and a slightly less satisfactory method has been employed to determine γ . Two of Higgins's curves for New Zealand soil have been chosen for purposes of illustration, the moisture contents in the two samples being very nearly equal, and it is assumed that γ will be the same for both. The constants of the soil samples necessary for calculations are set out below:

Soil	Moisture %	Porosity	Cylinder		Depth of injection cm.	Amount of injection
			Area cm. ²	Depth cm.		
New Zealand	50.98	0.56	84.5	15.2	15.2	12,920 mg.
(Volcanic)	51.51	0.53	83.6	30.4	15.2	(10 c.c.)

There is no record of temperature or barometric pressure during the experiments. The pressure at Rothamsted at the time of the experiments was 29.8 in., and no serious error will arise by neglecting the pressure correction. The temperature is a more vital factor and it is probable that most of the experimental scatter is due to temperature variations. The value used, 16° C., is taken as the mean laboratory temperature during the two or three days for which the experiments lasted. From the decay curve for the injection at the bottom (case i) the value of γ was found by logarithmic plotting. The steady state rate of emission was calculated, assuming the mean temperature to be 16° C. and using the value of D obtained from Fig. 2 for the given pore-space ($S=0.56$). The

¹ Private communication.

theoretical growth curve was then deduced, using equation (9 i a) on p. 446. The complete theoretical curve and the experimental points are shown in Fig. 4. For the results of the half depth injection experiment a slight adjustment is necessary. Assuming the same temperature, the steady state emission should be 12.8 mg./min.: the recorded value is 11.5 mg./min. This discrepancy is probably due to a downward flow of the liquid carbon disulphide at the time of injection so that the ideal

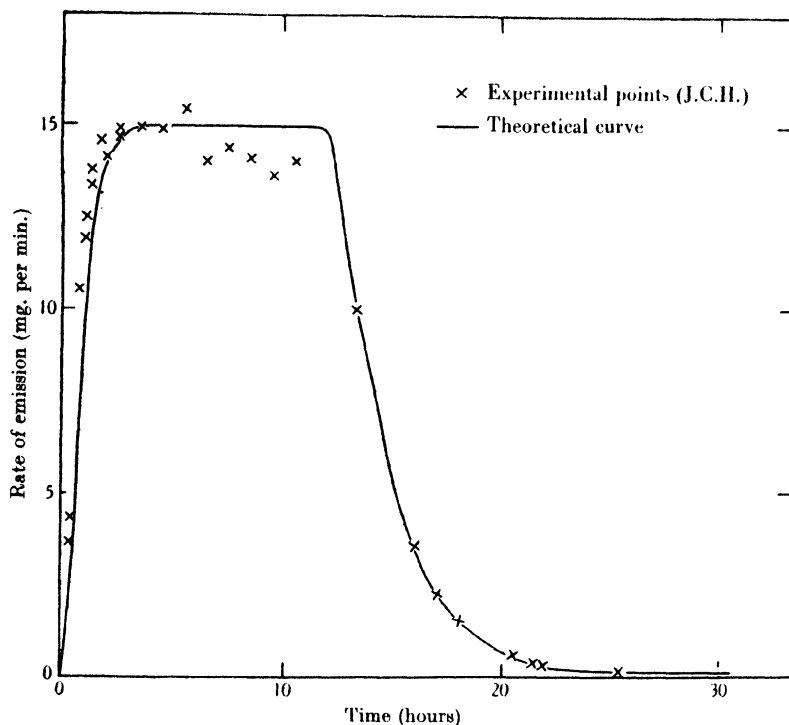


Fig. 4. Rate of emission of carbon disulphide vapour from surface of cylinder of soil 6 in. deep. Injection at bottom.

layer across the tube is not to be regarded as half-way down but some distance below, i.e. the steady gradient is less than it should be and the rate of emission is lower. The theoretical curve for this experiment has therefore been derived from an assumed steady state value of 11.5 mg./min., and the value of γ found from the previous curve. The theoretical curve and experimental points are shown in Fig. 5.

Considering the theoretical and experimental assumptions made, the agreement is good and indicates that given a knowledge of the adsorption

factor, the pore-space, and the temperature, the spreading of carbon disulphide vapour through soil can be quantitatively described. The principal assumption made, namely that γ is constant at all vapour pressures, is one which requires further experimental confirmation. The constant will vary with the moisture content of the soil and probably from soil to soil. In addition to γ being constant, the theory requires that the equilibrium between the two phases should be instantaneously attained. This may not be so and there may be a lag between the pressure changes and the attainment of equilibrium. Such a time lag would account for the phase difference between the observed and theoretical curves of Figs. 4 and 5. The amount is not very great and is probably

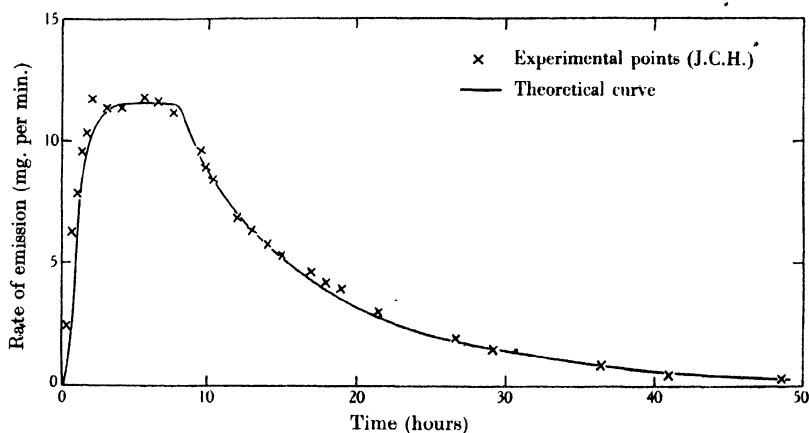


Fig. 5. Rate of emission of carbon disulphide vapour from surface of cylinder of soil 12 in. deep. Injection: 6 in. deep.

less than the differences which will be found in field soils arising from chemical and biological action, physical heterogeneity and temperature variations.

Examination of these adsorption results brings out one point which has an important bearing on the steady-rate experiments. For a soil sample little more than 1 in. thick the time taken to set up the steady state varies from 40 to 100 min., depending on the type of soil, pore-space and moisture content. In the case of the 6 in. columns of New Zealand soil the time is about 3 hr. and would probably be greater for smaller pore-space. It is obvious that reliable steady state measurements can only be made when this growth stage is ended: hence the neglect of the readings during the first 80 min. of the experiment quoted on p. 449. One can reasonably expect that carbon dioxide will behave in the same

way although the times may be different. In Buckingham's experiments a 4 in. column of soil was used and readings were taken after an interval of 20–30 min. It is highly probable that in no case had the steady state been attained when the observations were made and, as a consequence, that all values of the diffusion coefficient were under-estimated, the error being greatest for the smallest values of the porosity. Thus we have a qualitative explanation for the difference between the present steady state results and the results of Buckingham. As carbon dioxide is the gas about which information is most desired, some direct diffusion measurements will be made and the suggested explanation of the discrepancy tested quantitatively.

SUMMARY

The dependence of the coefficient of diffusion, D , upon the porosity, S , of a granular solid is investigated experimentally. For steady state conditions, using carbon disulphide and acetone vapours, it is shown that a curve connecting D/D_0 and S can be drawn which is independent of the nature of the solid, its moisture content and, within limits, its texture. For a limited range of values of S ($0.0 < S < 0.7$) a good approximation is $D/D_0 = 0.66S$ and over this range the diffusion coefficients are larger than those found by Buckingham for carbon dioxide.

Investigation of the non-steady state shows that in soils the attainment of pressure equilibrium is retarded by adsorption, and it is suggested that Buckingham's low values for steady-state conditions can be attributed to premature observations of the diffusion rates; the steady state had probably not been attained when his measurements were made.

ACKNOWLEDGEMENTS

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GAS AND VAPOUR MOVEMENTS IN THE SOIL

II. THE DIFFUSION OF CARBON DIOXIDE THROUGH POROUS SOLIDS

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(With Two Text-figures)

IN the first paper of this series (Penman, 1940; referred to below as I) it was suggested that the low values reported by Buckingham (1904) for the diffusion of carbon dioxide through soils could be attributed to his premature observation of the diffusion rates, the disturbing factor being the adsorption of gas by the soil, which retards the attainment of the steady state. Evidence of retardation was obtained in the experiments with carbon disulphide and acetone (I), and the work of Smith & Brown (1933), discussed below, provides indirect evidence of the same effect for carbon dioxide diffusing through soils. The simple and accurate technique devised for the vapours of carbon disulphide and acetone will not serve for experiments with carbon dioxide, but because of its great biological importance an attempt has been made to overcome the experimental difficulties disclosed by the results of earlier workers, and to obtain quantitative confirmation of the explanation advanced by the author to account for the discrepancy.

Buckingham's chief trouble was to produce and maintain a known partial pressure gradient across his soil samples without causing any difference in total pressure, and he expressed doubts as to whether his technique was always satisfactory. In the course of an attempt to improve on Buckingham's arrangement, Smith & Brown found that their soil samples were biologically active and producing carbon dioxide. In calculating diffusion coefficients a correction was made for the soil-produced carbon dioxide, but as far as it is possible to recalculate their results from the data supplied, the coefficients tabled appear to be too small ($\times 1/30$), suggesting some systematic arithmetical error in their calculations. Because of this and the need for a correction factor the numerical values of their coefficients will not be discussed, but attention is directed to one general feature of the results. "The rate of diffusion

increased with the length of the diffusion time in all cases except one. . . . Thus it would seem that the correction factor was inadequate."

This explanation is probably only partly true, and it is almost certain that Smith & Brown were getting experimental evidence of a source of error unsuspected by Buckingham, namely, adsorption of the gas by the soil. It is therefore obvious that the general nature of these results reinforces the conclusion already reached, viz. that for reliable measurements of the steady state dependence of diffusion upon porosity it is necessary to eliminate the effects of adsorption, either by using non-adsorbing materials or by delaying observations until the adsorption is complete. To avoid corrections for biological activity the granular materials must be biologically inert, and we must therefore avoid the use of soils when working with carbon dioxide. This is not a serious restriction, for we have seen that moist and dry soils conform to the general behaviour of other solids when carbon disulphide and acetone are used, and we assume that the same will be true for carbon dioxide.

A method of producing a partial pressure gradient of carbon dioxide has been devised and is described below. It is believed that it satisfies the condition that there must be no total pressure gradient, i.e. no viscous flow of an air-CO₂ mixture, but it suffers from one disadvantage. It is not possible to make a determination of the coefficient of diffusion of carbon dioxide into free air (D_0), and the value used in the calculations is $D_0 = 0.139 \text{ cm.}^2/\text{sec.}$ This is the mean of a number of values taken from standard works of reference, the individual values ranging from 0.134 to 0.142 $\text{cm.}^2/\text{sec.}$

THE APPARATUS AND METHOD OF OPERATION

The apparatus is conveniently described in three sections: (i) the reservoir, (ii) the diffusion apparatus, (iii) the measuring system.

(i) *The reservoir.* Instead of using Buckingham's method of producing a gas mixture by mixing streams from cylinders an automatic method has been used, based on the dissociation of sodium bicarbonate in solution. This proceeds until an equilibrium is set up between the concentrations of carbonate ion and dissolved carbon dioxide, the latter coming into equilibrium with the carbon dioxide content of the air above the solution. Ten litres of solution containing sodium carbonate (c. $M/10$) and sodium bicarbonate (c. $M/2$) were made up. In a closed system the air above the solution gradually attains an equilibrium, the carbon dioxide content being about $1\frac{1}{2}\%$ (by volume) at 25° C. The solution is

kept in two graduated 10 l. jars and, at the beginning of an experiment, one, the reservoir, will contain about $1\frac{1}{2}$ l. of solution. The air above this solution, presumed in equilibrium, can be displaced by running in liquid from the second jar, a delivery tube carrying the displaced mixture to the diffusion apparatus. About 7 l. are run in, thus leaving $1\frac{1}{2}$ l. in the second jar which then acts as the reservoir in the next experiment. A reservoir thus provides about 7 l. of a mixture of carbon dioxide and air for each experiment, and under the restricted conditions this is sufficient. The attainment of equilibrium is rather slow, and this simplified system has been slightly modified by introducing a third jar and a further 2 l. of solution, and limiting experiments to two per day, after which the jars are left overnight to come into equilibrium.

(ii) *The diffusion apparatus* (Fig. 1). The cylindrical holder previously employed is screwed into the tray of a tobacco tin which fits easily over the rim of a wide-necked bottle cut in half, a rubber gasket at the holder-tray junction and a rubber band at the tray-bottle junction making the joints gas-tight. Supported over the holder is a cylindrical collecting chamber with a clearance of 2 or 3 mm. at the bottom, sides and top. From the centre of the top of this chamber a tube leads away to the measuring system, and a side limb leads to an oil manometer. An aspirator behind the measuring system draws air up the sides of the holder and across the top of the granular solid in it, and any gas which diffuses through the solid from below is carried away in this air stream. To remove the normally occurring carbon dioxide from the air before it enters the collecting chamber, the upper part of the apparatus is loosely surrounded by a packing of Sofnolite (soda-lime+indicator). Blank experiments showed this to be quite efficient at normal rates of flow without causing any reduction of total pressure at the surface of the sample in the holder. Cleaning of the air in this or some other way is essential, as local variations in carbon dioxide content are sufficiently great to prevent reproducible results being obtained.

The space below the holder is closed by a rubber stopper carrying three tubes. The central one is connected to the delivery tube of the reservoir, and opens out conically to within about 1 cm. of the grid at the bottom of the holder. In this way the velocity of the gas-air mixture is reduced to negligible proportions and the incoming mixture is uniformly distributed below the grid. The second tube is a wide outlet tube (diameter $1\frac{1}{2}$ cm.) which carries away the gas mixture without any increase of total pressure in the bottle. A little Sofnolite in this tube takes out most of the carbon dioxide before the stream passes out into the

atmosphere. A thermometer in the outlet tube records the gas temperature. The remaining tube is connected to the other limb of the manometer.

With this design of apparatus, the manometer shows that there is no excess of total pressure below, and no deficit above the holder except at

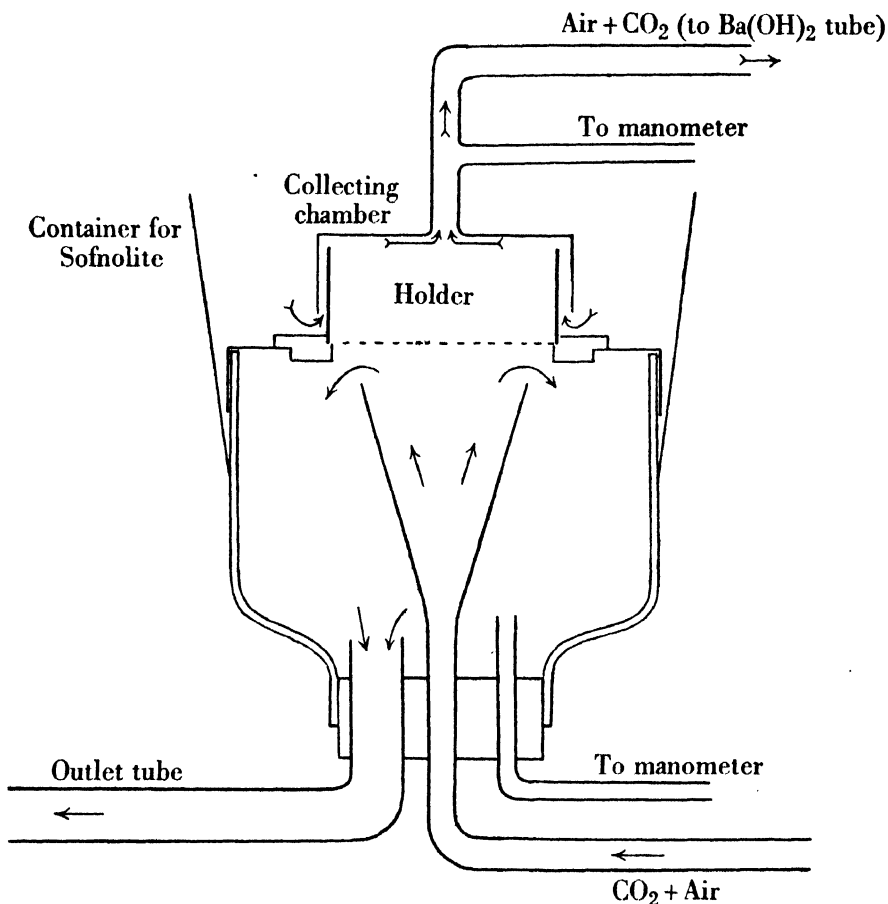


Fig. 1. Section of diffusion apparatus. (For the sake of clearness neither the granular solid nor the Sofnolite air filter is indicated.)

the instants of turning on the reservoir or aspirator taps. As a confirmation of the efficiency of the arrangement, results have been unaffected by changing the rates of flow.

(iii) *The measuring system.* The volume of air drawn through the diffusion apparatus is measured by the graduated aspirator. The air passes through one of two Pettenkofer tubes containing a measured volume of a solution of barium hydroxide and barium chloride. (The

stock baryta solution is almost saturated—*c. N/5*—and 10 or 15 c.c. are diluted to about 80 c.c. with CO₂-free water in the Pettenkofer tube.) The carbon dioxide is estimated by titrating the unchanged baryta with standard acid (*c. N/10*).

The method of operation is as follows. The holder is packed with the granular solid (sands, common salt, kaolin and kieselguhr, and mixtures of these have been employed) and weighed, the porosity being determined as previously described (I, p. 449). It is then replaced on the bottle, the rubber band slipped over the joint, and then the collecting chamber is clamped in position. Round the bottle is placed a bottomless split cardboard carton held in place by a rubber band, and this is filled with Sofnolite up to the level of the top of the collecting vessel.

One of the Pettenkofer tubes is filled with water and the other with the diluted baryta solution together with a few drops of thymol blue as indicator. At zero time the clock, the flow from the reservoir and the flow to the aspirator are started, the latter passing through the water tube. These conditions are maintained for a period varying from 20 to 60 min., depending on previous experience (I) of the particular material in the holder; at the beginning the rates of flow are adjusted and thereafter are kept as constant as possible. When the steady state is presumed to have been set up, the aspirator flow is redirected through the baryta tube by a two-way tap. To ensure complete absorption of the carbon dioxide a considerable excess of baryta is used, and in the experiments to be reported below the amount unconverted into carbonate was rarely less than three-quarters. The flow through the baryta tube lasts for a period between 60 and 20 min., at the beginning and end of which the temperature, aspirator and reservoir readings are noted. The other Pettenkofer tube is then washed out and a measured volume of baryta run in, diluted as before, connected directly to the reservoir, and a known volume of gas-air mixture is passed slowly through the baryta. The contents of the tubes are transferred separately to a suitable flask and titrated with acid previously standardized in terms of the stock baryta. Knowledge of the absolute concentrations is not essential for the calculation of the diffusion coefficient. From the first baryta tube the amount of carbon dioxide which has diffused is found, and from the second the concentration of carbon dioxide in the gas-air mixture, both expressed in terms of cubic centimetres of acid. The concentration difference causing diffusion depends upon both amounts. The higher pressure is reduced by the diffusion through the solid, and the lower pressure is given sufficiently closely by the concentration of the stream to the aspirator.

EXPERIMENTAL RESULTS

The experimental data must be corrected for the impedance of the grid and of part of the air below to obtain the effect of the solid alone. Calculation of the uncorrected value of the diffusion coefficient is shown immediately below for a typical set of data and is followed by a consideration of the correction for the impedance of the grid and lower chamber.

Mixture of sand and kieselguhr ($S=0.56$). Barometer: 29.8 in.

(i) Diffusion experiment.

Mean temperature = 22.5° C.

Baryta in test tube = 10.00 c.c. = 20.40 c.c. HCl.

Time: 40–70 min. = 1800 sec.

HCl for unconverted baryta = 16.40 c.c.

\therefore CO_2 diffused = 4.00 c.c. HCl.

Volume from reservoir = 2.57 l.

Volume to aspirator = 3.25 l.

(ii) Concentration experiment.

Baryta in test tube = 15.00 c.c. = 30.60 c.c. HCl.

Volume from reservoir = 1.13 l.

HCl for unconverted baryta = 21.20 c.c.

\therefore CO_2 absorbed = 9.40 c.c. HCl.

\therefore Concentration air- CO_2 mixture = $9.40/1.13$ c.c. HCl/l.

= 8.35 c.c. HCl/l.

Mean concentration on upper side of holder = $4.00/3.25$ c.c. HCl/l.

= 1.23 c.c. HCl/l.

Mean concentration on lower side of holder = $8.35 - (\text{amount diffusing per litre})$

= $8.35 - 4.00/2.57$ c.c. HCl/l.

= 6.79 c.c. HCl/l.

\therefore Concentration difference causing diffusion = $6.79 - 1.23$ c.c. HCl/l.

= 5.56 c.c. HCl/l.

The coefficient of diffusion (D) is defined by

$$dq/dt = DA (n_2 - n_1)/l \quad (\text{I, p. 439}),$$

where $(n_2 - n_1)$, the concentration difference producing diffusion, is measured in units of q per c.c. In the above we take as our unit of quantity the carbon dioxide equivalent of 1 c.c. of acid. A and l are as before (22.30 cm.^2 and 2.64 cm.), and hence

$$D' = (4.00 \times 2.64)/(22.30 \times 5.56 \times 10^{-3} \times 1800) \\ = 0.0475.$$

This value has to be reduced to standard conditions ($T=273^{\circ}$ K. and $P=30.0$ in.) and we obtain $D_{\text{un.}} = 0.0405$.

Correction for impedance of gauze and lower chamber. In the earlier paper (I, p. 441) the diffusion current (C) was considered as dependent upon two factors, the diffusion potential difference (E) and the impedance

(Z) of the material through which the diffusion took place. The total impedance, $Z + Z_0$, was considered in two parts: one, Z , due to the material in the holder, given by l/DA , and the other, Z_0 , including both the impedance of the gauze and of the air space between the bottom of the holder and the surface of the evaporating liquid. Using a fabric support on the grid the values of Z_0 were for carbon disulphide 0.78, and for acetone 0.88, the products $Z_0 D_0$ being 0.0803 and 0.0835 respectively, which should be the same for equal air columns beneath the holder. The mean value is 0.082, and the part contributed by the air column beneath the holder is given by the ratio of its depth to its area of cross-section. The reservoir used in I was 1.6 cm. deep, 34 cm.² cross-section, and with 7 c.c. liquid in it (0.2 cm. deep) the air column would be 1.4 cm. deep. Hence

$$D_0 Z_0 \text{ for the air column} = 1.4/34 = 0.041$$

$$\text{and } D_0 Z_0 \text{ for the gauze} = 0.082 - 0.041 = 0.041.$$

(The equality of these values is a coincidence.) This value will hold for the present apparatus, and to it must be added an amount depending on the position of the layer of the carbon dioxide-air mixture from which diffusion takes place (corresponding to the surface of the liquid in the earlier apparatus). The effective position of this layer lies somewhere between the bottom of the gauze and the top of the conical delivery tube, and we assume that it is midway between the two. The separation is 0.95 cm. and the area of cross-section of the bottle is 40 cm.², and hence the value of ZD for the space below the gauze is $0.475/40 = 0.0119$, and the total for air space and gauze is thus $0.0410 + 0.0119 = 0.0529$. Taking D_0 for carbon dioxide as 0.139 cm.²/sec., we obtain $Z_0 = 0.0529/0.139 = 0.381$. If Z is the impedance of the solid in the holder (for which $l/A = 0.1184$),

$$D = 0.1184/Z,$$

$$D_{\text{un.}} = 0.1184/Z + Z_0,$$

$$\begin{aligned} \therefore 1/D &= 1/D_{\text{un.}} - Z_0/0.1184 \\ &= 1/D_{\text{un.}} - 3.21. \end{aligned}$$

For the particular case for which $D_{\text{un.}} = 0.0405$,

$$1/D = 24.70 - 3.21 = 21.49,$$

$$\therefore D = 0.0465, \text{ i.e. } D/D_0 = 0.335.$$

If the layer of maximum concentration is assumed to be at the top of the conical tube or at the bottom of the gauze the value becomes 0.048 or 0.045.

The value of D may therefore be in error by $\pm 3\%$ at $S = 0.56$. The

possible error increases with S and will be $c. \pm 1.35\%$ at $S=0.21$ and $\pm 5.6\%$ at $S=0.87$. This source of error, if effective, will affect all results in the same sense; superimposed will be the random experimental errors arising from the measurement of volumes (reservoir and aspirator) and from the titrations (diffusion, concentration, and baryta-HCl check). As far as possible these have been eliminated by performing several experiments on each sample with different rates of flow and for different lengths

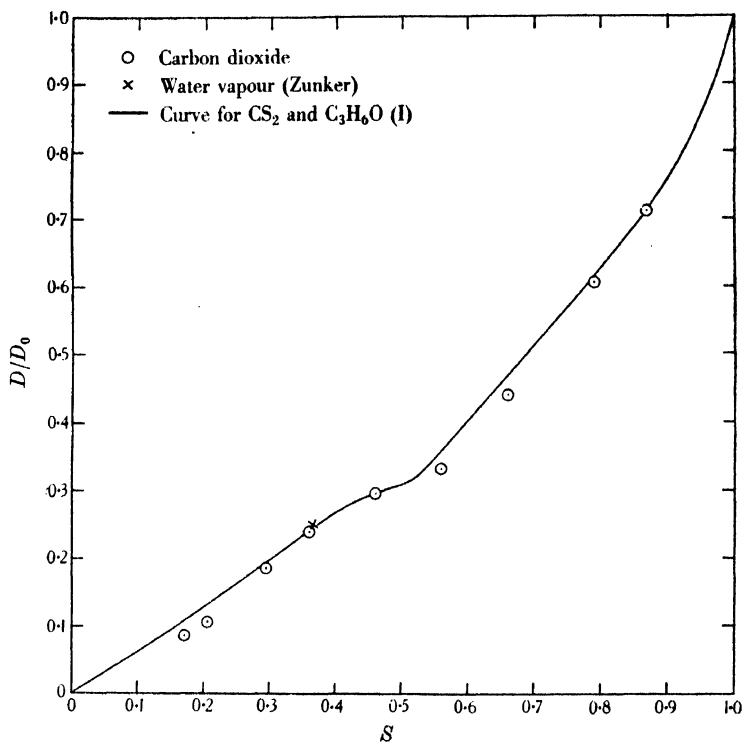


Fig. 2. Dependence of coefficient of diffusion on porosity

of time, and each point on the curve represents the mean of three or four determinations. The range of granular solids used is not as extensive as that used in I, and only those solids have been chosen which were known to come quickly to the steady state in the carbon disulphide experiments. In general, the sands were allowed about 20 min. to attain the steady state and the experimental run lasted for an hour, and for the more finely divided materials, such as kieselguhr and kaolin, the periods were reversed.

Sufficient experimental points have been obtained to permit a fair comparison of the results with those for carbon disulphide and acetone,

In Fig. 2 have been plotted the values of D/D_0 , the curve obtained for carbon disulphide and acetone, and also an experimental point for the diffusion of water vapour. This has been calculated from the results, given by Zunker (1930), of experiments on the movement of water vapour through a column of sand. Although interpreted by Zunker in terms of viscous flow of the vapour, the conditions of the experiments were purely diffusive, and we may use the results to give an indication of the way in which water vapour conforms to the general behaviour.

DISCUSSION OF RESULTS

The points for carbon dioxide lie on or near the curve, and remembering that the present method of experiment cannot be as accurate as that used in I, which involved a weighing only, we conclude that the curve obtained for the vapours also holds for carbon dioxide diffusing through dry solids which are biologically inactive. In the experiments with carbon disulphide it was shown that moist and air-dry soils fitted into the same scheme, and it is reasonable to assume that in the steady state the diffusion of carbon dioxide through soils will depend upon the porosity in the same way, i.e. over the range of technical interest ($0.0 < S < 0.6$), we may use the relation $D/D_0 = 0.66S$. In field soils it is exceedingly unlikely that a true steady state will ever be set up, because the centres of biological activity will, in general, be irregularly distributed in the soil and be of variable intensity, and the readjustment of partial pressure gradients will be retarded by the effects of soil moisture and adsorption in all soils, and also by chemical action in soils which contain an appreciable quantity of carbonate. The retardation will tend to smooth out the effects of small fluctuations in biological activity, but in the absence of direct knowledge of the nature of the adsorption equilibria between soil and gas one cannot say what is to be regarded as a "small" fluctuation. As long as the fluctuations are small a pseudo-steady state will be maintained; when the fluctuations are large the interpretation of the rates of surface emission of carbon dioxide in terms of soil activity will be very complex. The existence of large fluctuations may be the reason why some authors (e.g. Smith & Brown, 1931, 1932) have suggested that some other mechanism in addition to gaseous diffusion is needed to account for the irregular day to day variations in surface emission of carbon dioxide. To the complexity already noted there must be added the effects of changes in the environment. Changes in the porosity due to wetting or drying, and changes in the temperature of the soil, will modify the coefficient of diffusion; they will also modify the

gas-condensed phase equilibrium (using the term condensed phase to include dissolved, adsorbed and chemically bound gas), and it is therefore somewhat unreasonable to expect a simple diffusion theory to give an exact account of carbon dioxide movements in soil. The most that one can ask is that it should account broadly for the normal process of aeration. In the introductory survey of I we saw that even on the basis of Buckingham's equation the amount of diffusive flow was sufficient to account for normal aeration without invoking meteorological factors: the present account shows that his theoretical predictions were considerably underestimated.

As indicated in I, the materials to which this discussion is applicable are granular solids whose particles approximate to spheres and in which the distribution of pore space is isotropic. As the pore space in a soil is decreased by wetting, it is very probable that the isotropy will cease to exist; small air pockets may be formed which are completely isolated by liquid or solid from other parts of the soil atmosphere, and these will be ineffective as diffusion channels but still contribute to the pore space. It is to be expected, therefore, that the curve will become less reliable as the pore space decreases, and the backward extrapolation to $S=0.00$ from $S=0.15$ is to be regarded as indicating the order of magnitude of the diffusion coefficient. The actual value may be greater or less. It will be greater when the pore space is provided by vertical cracks in the soil, and will be less in cases where parts of the pore space do not contribute to the system of through air channels. This lack of precision in the application of diffusion theory to the aeration of wet soils will apply with equal force to any other mechanism which is postulated.

To make a quantitative survey of the effect of diffusion on aeration we make use of the relation $D/D_0=0.66S$, which was shown in I to represent the experimental results with sufficient accuracy over a considerable range of porosity. Let us assume that we have a uniform concentration gradient of carbon dioxide down to a depth of l cm., the concentrations (c.c./c.c.) being c_1 and c_2 at l and the surface respectively. Consider the emission of gas from an area A cm.² of the soil surface, the porosity being S and the mean temperature T° K. For the present purpose the pressure correction may be ignored. The volume of carbon dioxide passing out in time t (sec.) is given by

$$\Delta V \text{ (c.c.)} = t \times 0.66 D_0 S (T/273)^2 \times (c_1 - c_2)/l.$$

The total volume of carbon dioxide in the prism ($l \times A$) is

$$V = lAS \times (c_1 + c_2)/2,$$

$$\therefore \Delta V/V = 1.32t D_0/l^2 \times (T/273)^2 \times (c_1 - c_2)/(c_1 + c_2).$$

As a first approximation, if c_2 is small compared with c_1 , this becomes

$$\Delta V/V = 1.32t D_0/l^2 (T/273)^2,$$

which is independent of the concentration and the porosity. The significance of this equation is shown most conveniently by determining the time required to renew the air content of the soil, i.e. by setting $\Delta V/V=1$ and finding t . Taking $T=273+15$, and $D_0=0.139$ cm.²/sec., we find $t=l^2 \times 1.12 \times 10^{-3}$ hr. For $l=30$ cm., $t \doteq 1$ hr., i.e. the air to a depth of 30 cm. will be completely renewed every hour. Where c_2 is not negligible, the period is increased by a factor $(c_1+c_2)/(c_1-c_2)$, and taking for illustration some data given by Russell (1937, p. 485) for arable land, (a) unmanured ($c_1=0.2\%$, $c_2=0.03\%$), and (b) dunged ($c_1=0.4\%$, $c_2=0.03\%$), the values of t become

$$t_a=1.35 \text{ hr.}; \quad t_b=1.16 \text{ hr.}$$

For $l=20$ cm., these values will be reduced to $4/9$ and t_a ($l=20$) is 0.60 hr. This time may be compared with the finding of Romell, quoted by Russell (1937, p. 486), from which it was estimated that the soil air must be completely renewed every hour to a depth of 20 cm. to keep it at its normal composition. The times are of the same order, and we may summarize the effect of diffusion on aeration as follows. The physical conditions in the soil atmosphere are such that diffusion of carbon dioxide upward must be taking place and this movement may be accelerated by meteorological changes. The process of diffusion will be continuous, and in amount it is sufficient to account for the observed rates of soil respiration. Although an occasional favourable combination of meteorological changes may also be sufficient, such a combination is not a necessary condition for the promotion of aeration.

One further point remains to be discussed, bringing us back to one of the basic assumptions, namely, that the diffusion curve is unique. This *a priori* assumption has been confirmed for carbon disulphide, acetone and carbon dioxide, and a single point for water vapour conforms to the general behaviour. An application of this unique dependence of D/D_0 on S has been made (Penman & Schofield, 1939) to estimate the extent of water movement in soils as vapour under a vapour-pressure gradient arising from temperature differences. Even under conditions much more extreme than any likely to arise in practice, it was shown that the transfer was very small and that distillation under a temperature gradient is a negligible factor in soil water movements.

SUMMARY

Apparatus for measuring the rate of diffusion of carbon dioxide through granular solids is described and the results obtained with it are shown to conform to the curve connecting D/D_0 and S previously obtained for carbon disulphide and acetone. The equation $D/D_0 = 0.66S$, which it is suggested should replace Buckingham's equation $D/D_0 = S^2$, is applied to a discussion of soil aeration, and it is shown that at all porosities the rate of diffusion of carbon dioxide from the soil is sufficient to account for normal respiration without invoking the assistance of meteorological changes. A further application of the equation to water vapour movement in soils is briefly discussed.

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METEOROLOGICAL AND SOIL FACTORS AFFECTING EVAPORATION FROM FALLOW SOIL

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SUMMARY

Using the conception of natural periods, for which the difference between rainfall and drainage can be equated to the evaporation, the mean daily rates of evaporation from a block of fallow soil at Rothamsted are examined for 94 periods varying in length from 13 to 45 days. In seeking correlations with single daily meteorological observations two types of treatment are employed. (1) The year is considered in three seasons of four months each—summer, winter and two equinoctial pairs of months—and it is shown that an almost complete description of evaporation can be obtained in terms of rainfall only, the nature of the correlation varying from season to season. (2) A general treatment is attempted in physical terms, considering evaporation as due to diffusion across a non-turbulent boundary layer whose thickness is determined by wind velocity, the soil surface being assumed to be continuously at 100 per cent R.H. The general agreement between observed and predicted values is very good in winter. The summer data are shown to lie between the theoretical limits imposed by the assumptions of (i) continuous 100 per cent R.H. at the surface, and (ii) a steady retreat of the 100 per cent R.H. layer into the soil, i.e., no upward movement of *liquid* during the evaporation process. The considerable scatter in the data is attributed partly to the inadequacy of single daily meteorological observations but chiefly to the lack of knowledge of the conditions existing at the soil surface.

The data on which this survey is based are the 09.00 h. meteorological readings at Rothamsted and the daily totals of rainfall and drainage as recorded by the 1/1,000 acre gauges installed by Lawes and Gilbert in 1870. The soil of the drain gauges is in its natural undisturbed state, fallow and uncultivated. Since 1870 there have been several papers dealing with different aspects of the drainage data and these are discussed in a general survey of the records (Penman and Schofield, 1941); a brief recapitulation of the relevant parts of this survey will serve as a basis for the present account.

One of the main objectives was the establishment of the conditions under which the difference between the total rainfall and the total drainage of a selected period—generally defined as the "deficit" of the period—can be equated to the evaporation which has occurred in that period. The conditions are satisfied for a "natural" period, which is the interval between the cessations of two falls of rain that cause drainage, the total drainage being

measured between the two cessations of percolation. Hence the drainage period is slightly out of phase with the rainfall period owing to the time taken for complete discharge, and for accurate demarcation of natural periods it is essential that each fall of rain shall be followed by at least one rainless day. This does not always occur, and for greater convenience in observation the practical natural period is taken as the interval between two cessations of drainage, or between two well-marked drainage minima. This introduces two end errors which tend to annul each other and the resultant error is no greater than that involved in taking one day as the unit of time. No actual natural periods were examined—that is one of the present objects—but the conception was applied to consider how far arbitrary periods could be regarded as approximating to natural periods. Satisfactory arbitrary periods were considered and the conclusions drawn from them were:—

(a) Civil Years. Annual evaporation (1881-1934) is nearly constant, increasing slightly with annual rainfall;

(b) Half Years. (i) Winter (Oct.-March inclusive) evaporation is constant and independent of rainfall. There is some scatter in the data which is not due to variation in mean air temperature;

(ii) Summer (April-Sept. inclusive) evaporation is about twice as great as that for winter and shows marked dependence on summer rainfall. The mean curve for the data is approximately parabolic with unit slope near the origin. Part of the small scatter in the data is attributed to the effects of rainfall distribution in summer seasons;

(c) Long period mean monthly totals. The twelve 50-year means are uniquely dependent upon the "evaporating power" of the air, which is, in effect, the mean saturation deficit in mm. of mercury. A similar plot against mean air temperature yields a loop with spring values about 50 per cent greater than autumn values at the same mean temperature. The winter data agree with estimates made for evaporation from an open water surface, but the summer values fall below the open water curve, and are nearly constant.

Individual calendar months were rejected as unsuitable for the following reasons:—

(1) Rain or snow falling near the end of one month may contribute to the drainage of the next, i.e., the measured monthly deficits are over- and under-estimates of the evaporations in the respective months;

(2) The evaporation occurring during a rainless period at the end of one calendar month will be included in the deficit of the next, i.e., the evaporations will be under- and over-estimated respectively;

(3) In case (2) a further source of error arises when correlations are made. The deficit of the second month is due to the evaporation of the second month and part of the first; for correlation purposes the meteorological conditions should be averaged over the same period and not over the second month only.

In a statistical note following the general survey, Sahni (1941) examined short natural periods of two to thirteen days in June, July and August, and concluded that a cubic regression on rainfall

accounted for most of the variance in evaporation, the residual variance being non-significantly correlated with mean wind velocity and relative humidity, the signs of the coefficients being as expected.

The purposes of the present account are:—

- (i) to extend the examination of evaporation to longer natural periods in all seasons of the years;
- (ii) to attempt to obtain greater precision in evaluating the "evaporating power" of the air so that a physical interpretation rather than an empirical statistical correlation can be employed;
- (iii) to see how far single daily meteorological observations can be used to estimate the evaporating power; and
- (iv) in the light of the deficiencies thereby exposed, to indicate what supplementary observations may be needed.

SEASONAL EVAPORATION AND RAINFALL

Ninety-four natural periods varying in length from thirteen to forty-five days and covering the years 1927-1939 have been con-

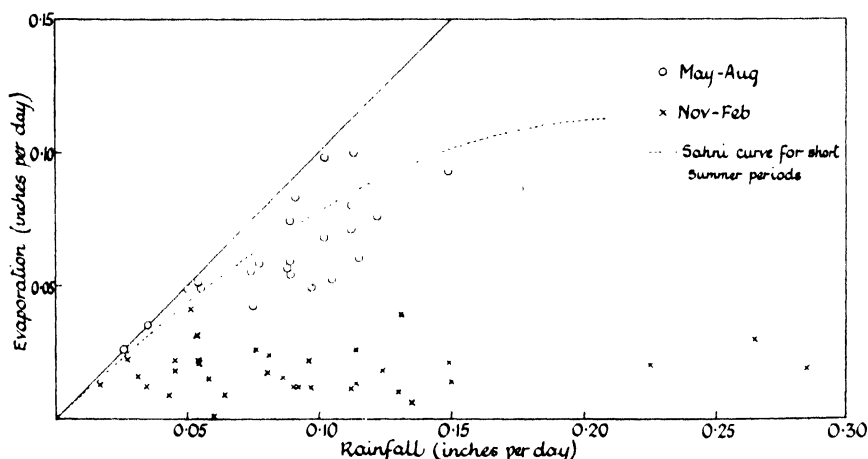


FIG. 1.—Dependence of seasonal evaporation on rainfall.

sidered. The mean daily rates of evaporation are shown in Fig. 1 plotted against the mean daily rainfall. To show seasonal effects the year has been split into three groups of four months and to avoid confusion only two sets of data are plotted. They are summer (May-Aug. inclusive) and winter (Nov.-Feb. inclusive). The points for periods falling in the equinoctial pairs of months overlap those for the summer and winter periods. The dotted curve is that obtained by Sahni for shorter summer periods, and, considering the almost four-fold extrapolation, the agreement is good. The main features of the figure correspond to those noted above (b, (i) and (ii)) for half-years. The winter data show no obvious dependence on rainfall; the summer data show a positive correlation with rainfall, the values asymptotically approaching the line

of unit slope near the origin. No points can lie above this line because for a natural period the deficit cannot exceed the rainfall.

The causes of the scatter of the points will be considered later. The curves show that if one is prepared to regard the year as built up of two or three seasons, the main features of evaporation can be interpreted in terms of regressions on seasonal rainfall alone, with a different regression for each season. To unify the data some factor allowing for seasonal variation in evaporation at constant rainfall is needed. The obvious choice is mean air temperature, and while the analysis of Crowther (1930) showed that mean seasonal temperature varied in phase with seasonal evaporation, that of Koshal (1934), confirmed by Sahni, showed that variations in mean air temperature were quite ineffective in accounting for intra-seasonal fluctuations such as are represented in the scatter of the points of Fig. 1.

It was the need for some other seasonal factor which led Penman and Schofield to attempt an approximate explanation in terms of the physical factors involved, and in the following section this approximate treatment is amplified.

EVAPORATING POWER

The process of evaporation is regarded as due to molecular diffusion of water vapour across a non-turbulent boundary layer immediately above the soil surface, the thickness of which is determined by the wind velocity, into a turbulent atmosphere where the diffused vapour is carried away. The movement of water to the soil surface may be as liquid or vapour. In the former case, if the supply is sufficiently rapid, the surface may be kept at 100 per cent R.H. and evaporation will proceed at a rate which is dependent only on air conditions. In the latter case, where the saturated layer is below the surface, the soil itself will impose a resistance to vapour movement and the rate of evaporation will be correspondingly reduced, and will be dependent on both soil and air conditions. The evaporating power is taken to correspond to the first case and in attempting a quantitative estimate the following assumptions are made:—

- (i) The surface of the soil is kept at 100 per cent R.H.;
- (ii) The soil surface temperature has the same value as the air temperature in the screen;
- (iii) The daily mean for each is given by the mean of maximum and minimum air temperatures;
- (iv) The mean daily humidity is given by the 09.00 h. reading;
- (v) Mixing of diffused water vapour is so complete in the turbulent layer that the R.H. immediately above the boundary layer is given by the value in the screen;
- (vi) The thickness of the boundary layer is determined by the wind velocity estimated from the Beaufort reading at 09.00 h. The rate of evaporation per unit area is then given by

$$D(p_1 - p_2)/760 \text{ l. cc. vapour per second,}$$

where D is the coefficient of diffusion of water vapour into air
 $= 0.24 \text{ cm.}^2/\text{sec.}$ at 10°C.

$p_1 - p_2$ is the partial pressure drop across the boundary layer
 $= (1 - \text{R.H.}) \times \text{saturation V.P. at air temperature.}$

l is the thickness of the boundary layer $= 60/V \text{ cm.}$ where V
 is the wind velocity in cm./sec. (see e.g. Brunt, 1934).

The mean Beaufort figure for the 94 periods is 2.00, which has been taken as equivalent to 240 cm./sec. Hence the mean value of l is 0.25 cm. , and making the necessary conversion of units, the mean daily rate of evaporation becomes

$$3.48 \times 10^{-2} \times (p_1 - p_2) \times B / 2.00 \text{ in. per day}$$

where $(p_1 - p_2)$ is in mm. of mercury and B is the mean Beaufort number for the period examined.

CORRECTION OF OBSERVED EVAPORATION FOR RAINFALL

The evaporating power is serving a double function. In the first place, it is being used to distinguish the members of the family of curves which could be drawn for different seasons as in Fig. 1; in the second, when the assumptions of the preceding section are satisfied it is being used to give an estimate of the actual evaporation. *A priori* we know that in summer the first of these assumptions will only be reasonable for a short time after a fall of rain and the evaporating power will represent the upper limit of possible evaporation.

In using evaporating power as a parameter distinguishing the seasonal regressions on rainfall it is necessary to standardise the rainfall conditions, and in the absence of any formal guide it is proposed that the observed evaporation be corrected to that for a rainfall of 0.08 in. per day. This is a fifty year mean value. The correction factors have been obtained from the regression equations of Koshal connecting monthly drainage and monthly rainfall. Recast, these fall into the form

$$R - D = a + bR', \text{ or } \Delta(R - D) = b\Delta R',$$

where $\Delta R'$ is the deviation from the mean rainfall. The constant b varies cyclically throughout the year from a December minimum of 0.015 to a July maximum of 0.50. In the absence of a diagram showing the measured evaporation before correction the general effect can be seen by comparing Figs. 1 and 2. In the former, the range of summer data is from 0.026 to 0.100 in. evaporated per day; in the latter, the range of the same data, corrected, is 0.038 to 0.091 in. per day. The correction has little effect on equinoctial data and practically none on winter data.

The results plotted in Fig. 2 are for all the available data, the three types of season being distinguished. Again temporarily ignoring the scatter, we can follow the seasonal changes in evaporation and note that for a considerable part of the year the points lie near the line of unit slope, confirming the inference from the earlier study that for the period October to March the evaporation from fallow soil is the same as would occur from an open water surface under the same meteorological conditions. As anticipated, the summer points lie below the line of unit slope, indicating that

under the more severe drying conditions the supply of water to the soil surface has not been sufficiently rapid to keep the surface at 100 per cent R.H.

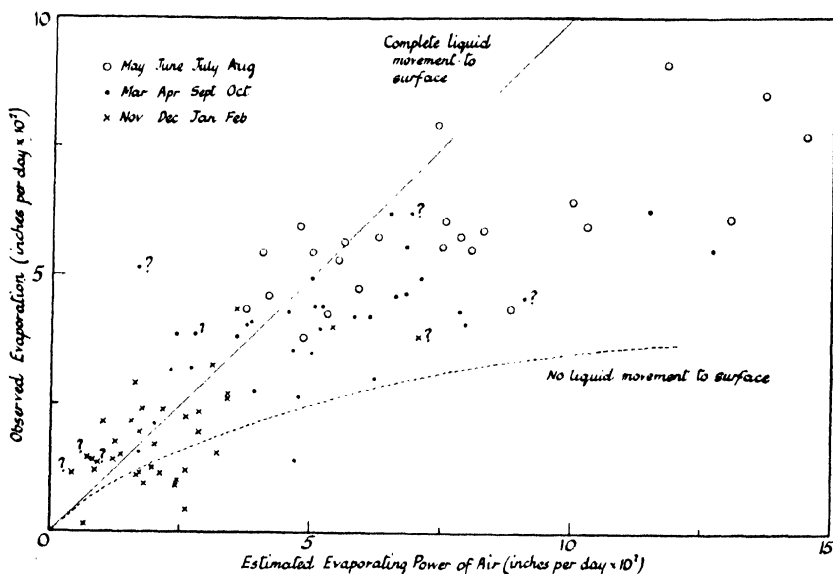


FIG. 2.—Seasonal evaporation from fallow soil (Rothamsted).

EVAPORATION IN THE ABSENCE OF LIQUID MOVEMENT TO THE SURFACE

The evaporating power of the air sets an upper limit to seasonal evaporation. A lower limit can be found by assuming that soil effects exert their maximum influence, and in the following very approximate treatment an estimate of the order of magnitude is made. It is assumed that no liquid movement upward occurs and that the layer at 100 per cent R.H. retreats within the soil as the evaporation proceeds. The process is then doubly hindered; (a) by resistance in the soil, and (b) by resistance in the boundary layer. The impedance of the soil (Z_s) is given by

$$Z_s = x/0.66 SDA \quad (\text{Penman, 1940})$$

where x is the depth of the layer at 100 per cent R.H.;

S is the fractional pore-space of the soil;

D is the coefficient of diffusion into air;

A is the area of cross-section;

0.66 is an experimentally determined constant.

For the air, $Z_a = l/DA$ as before, and the instantaneous rate of evaporation becomes

$$(p_1 - p_2)/760 (Z_a + Z_s) \text{ cc. vapour per sec.}$$

As evaporation proceeds the value of x increases, i.e., the instantaneous rate decreases. Taking S as 0.5 and using the mean value of l (0.25 cm.) and, integrating the equation set up, we obtain

$$0.126 E^2 + 1.04 E - 3.48 P = 0$$

where E = total evaporation in units of 0.01 in. in τ days;

P = product of τ and $(p_1 - p_2)$;

i.e., E/τ is the mean daily rate of evaporation for a period of τ days.

In a natural period the soil may be re-wetted several times by rain. We assume that the effective number of such re-wettings is given by the number of days on which the precipitation exceeds the mean daily evaporation for the period and that the value of τ is obtained by dividing the number of days in the period by the number of such "wet" days. Table I shows the seasonal variation of τ and $p_1 - p_2$, the means being taken by months, so that for each month a value of P is known and the twelve solutions of the quadratic can be found. From these the mean daily evaporations follow and they are plotted on Fig. 2 against the corresponding values for zero soil impedance. The actual points are not shown as they lie on a fairly smooth curve, a result not implicit in the analysis.

TABLE I

Month	Mean τ (days)	Mean $(p_1 - p_2)$ (mm.)	P	E	Evaporation per day (in.)	Evaporating power of air (in. per day)
1	1.85	0.57	1.05	2.5	1.35×10^{-2}	2.00×10^{-2}
2	2.3	0.77	1.75	3.8	1.65	2.70
3	2.7	1.28	3.45	6.2	2.30	4.50
4	2.8	1.72	4.80	7.8	2.80	6.00
5	2.95	2.22	6.55	9.6	3.25	7.75
6	3.85	3.02	11.60	13.7	3.55	10.60
7	3.3	2.08	6.85	9.8	3.00	7.30
8	3.1	2.22	6.85	9.8	3.15	7.75
9	2.8	1.60	4.50	7.5	2.70	5.60
10	2.7	1.10	2.95	5.6	2.05	3.85
11	1.65	0.62	1.00	2.45	1.50	2.15
12	1.9	0.53	1.00	2.45	1.30	1.85

There are so many assumptions involved in the derivation of the curve that it can only be claimed as representing the order of the daily evaporation to be anticipated in the absence of liquid movement to the surface. Except near the origin it lies below the observed points, and this is still true of the data before the rainfall correction is applied.

CAUSES OF SCATTER IN DATA

(a) General

The main sources of error lie in the approximate nature of the estimates of evaporating power. Accepting the mean of maximum and minimum air temperatures as the daily mean air temperature, it will not in general represent the mean soil surface temperature. The error in winter may easily amount to 0.5°C. or more, which will cause an error of 0.7×10^{-2} in. per day in the estimated evaporating power. Accepting this amount of tolerance on either side of the line of unit slope, most of the scatter can be taken up. In summer the effect of radiation will be to raise the soil surface temperature above the air temperature and the soil mean will generally be greater than the air mean. The evaporating power of the air will thus be an under-estimate of the potential

evaporation from the soil surface, but as the evaporation is also dependent upon soil conditions large variations in air conditions may have little effect on the observed evaporation. In the intermediate seasons cooling of the surface by evaporation and heating by radiation may again cause differences in mean temperatures sufficient to account for much of the scatter of the points.

The 09.00 h. readings of humidity will not be the same as the daily means; the error will not be the same in all seasons, but in magnitude it is probably not as important as that due to temperature.

The wind data must be similarly criticised. For precision anemometer readings should be used. The Beaufort readings have been used here for three reasons.

- (i) The rain-gauge enclosure is $\frac{1}{4}$ mile away from the wind recorder on the laboratory roof.
- (ii) The enclosure is somewhat sheltered to the south and west by a belt of trees 30 yards away.
- (iii) The drain-gauges have a stone coping 10 cm. high which will act as a wind break.

These last two reasons considerably reduce the accuracy of the estimate of the thickness of the boundary layer, and the scatter of the points in Fig. 2 is little worsened by using the mean value of l for all points. In view of the uncertainty, points with mean Beaufort numbers less than 1.3 or greater than 3.0 have been queried; these may be legitimately disregarded in making the comparison between observed and predicted values of daily rates of evaporation.

(b) *Particular*

(i) *Winter.* Many winter periods include snow falls. Drifting may cause unequal distribution so that the recorded "rainfall" is not always a true measure of the actual precipitation on the surface of the drain-gauge. Frost may hold up drainage near the end of a natural period thus leading to an error in the estimation of the evaporation.

(ii) *Summer.* The main cause of the summer scatter probably arises from differences in rainfall distribution in time. A fall of 0.8 in. of rain in one day rarely fails to produce drainage; the same amount spread over 10 days in summer is generally completely evaporated, and the more often the soil is rewetted the greater is the amount of summer evaporation. In general this means that the greater the total summer rainfall the greater will be the summer evaporation (Fig. 1), but in periods of equal totals and with other factors equal there will be variations in summer evaporation depending upon rainfall distribution. The most favourable case will be that in which the showers fall at intervals frequent enough to keep the soil surface wet; the observed points will then lie on the line of unit slope. An extreme case of formal interest is that in which rain falls continuously. Presumably no evaporation could occur and the curve of Fig. 1 would meet the rainfall axis at the appropriate value of the mean daily rainfall.

DISCUSSION AND CONCLUSIONS

This section follows the order of the points outlined on p. 403 above:—

(i) The results of this survey show that the main points established by Penman and Schofield for long arbitrary periods approximating to natural periods also hold for natural periods. A series of seasonal empirical relationships between evaporation and rainfall can be established (Fig. 1). In winter the water supply from below is sufficiently rapid to keep the soil surface at 100 per cent R.H.; the rate of removal is slow and the frequency of re-wetting by rain is high, particularly in November, December and January (Table I). Consequently, further winter rain has no effect on the surface humidity and hence none on the evaporation rate, so that winter evaporation depends only on the evaporating power of the boundary layer and is independent of total rainfall. The main cause of variance is the relative fluctuations of soil and air temperatures. In summer the upward movement between showers is not sufficient to maintain saturation at the surface and part of the resistance to evaporation is now due to the soil. Although some upward movement does take place the maintenance of summer evaporation is very dependent on re-wettings by rain, and, as Table I shows, these are less frequent than in winter. Hence we find that summer evaporation is markedly dependent on rainfall and on the way in which that rainfall is distributed in time; also that as the evaporating power of the air increases, the observed values depart more and more from the theoretical and begin to approach the values which they would have if soil resistance was at its maximum. From this obvious importance of soil factors in midsummer it is apparent that attempts to correlate summer evaporation with *air* conditions only will lead to results which may be seriously misinterpreted.

(ii) and (iii) The physical concepts used in deriving the evaporating power could doubtless be made more precise, but the greatest gain would follow from increased precision in the measurement of the quantities used to evaluate the evaporating power. If the suggested reasons for the scatter are accepted as reasonable we may regard the physical basis as adequate, i.e., it is possible to obtain a value of the potential evaporating power of the air from first principles involving no empirical correlations. Fig. 2 shows that the observed rate of evaporation for a considerable part of the year is of the same order as the evaporating power, the departures from expectation being randomly distributed about the line of unit slope, a very satisfactory result. For the warmer part of the year the potential evaporating power of the air ceases to be a measure of rate of evaporation owing to the increased importance of soil factors. The position of the dotted curve of Fig. 2 confirms the anticipation that in practice there is some movement of liquid and some movement as vapour when surface evaporation is in progress. It is not yet possible to give a quantitative account of the balance between these two types of response to a moisture gradient and it is hoped that further

evaporation studies will throw some light on this important problem in soil physics.

The inadequacy of single daily meteorological observations has been shown in discussing scatter. The chief sources of scatter are thought to be the non-equality of soil and air temperatures, the crude estimate of wind, and the effect of rainfall distribution. The last might be allowed for statistically and it may be that in this case some empiricism will be necessary; an alternative is suggested in the following section. Of the other two factors, the first arises because we have imposed a property on the maximum and minimum air temperature readings which is unreasonable; their mean cannot be expected to give the soil surface mean and the way out of this difficulty is obvious. The estimation of wind remains as a difficulty. The present use of the rainfall and drainage records was probably not envisaged when the site of the gauges was chosen, and under other conditions it would be possible to ensure that wind-breaks formed by trees were non-existent. The coping cannot be omitted as it is needed to prevent run-off and the washing away of surface soil, and it appears that to decrease the wind error we must introduce errors in estimation of the drainage. An effective compromise may be possible and we cannot say that the existing conditions, in the absence of the trees, are the best possible.

(iv) With this reservation the necessary improvements in observations are obvious. The substitution of daily means, taken from continuous records, for 09.00 h. readings would bring extra precision. The soil measurement chiefly needed is that of the actual water vapour pressure at the soil surface, to be compared with that 0.25 cm. above the surface. The difficulty is apparent; but if overcome, the measurement would enable evaporation in all seasons to be predicted. Failing this, the less difficult alternative is to measure the soil surface temperature from which exact prediction will be possible in winter, but empirical corrections for rainfall will be needed in summer.

ACKNOWLEDGMENT

The task of selecting natural periods and totalling up the rainfall, drainage, temperature and relative humidity for each was performed by Mr. P. Sahni, M.Sc., in the course of collecting data for his survey (referred to in the text). Having access to this material has considerably reduced the amount of computation involved in preparing the present account and my grateful thanks are offered to him for this valuable assistance.

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DRAINAGE AND EVAPORATION FROM FALLOW SOIL AT ROTHAMSTED

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(With Nine Text-figures)

THE three drain gauges at Rothamsted were built in the summer of 1870. As details of their construction were given by Lawes *et al.* (1881) and by Miller (1906), it is sufficient here to recall that they each enclose a block of undisturbed soil $1/1000$ acre (4 m.^2) in area kept free of vegetation; the three blocks of soil are respectively 20 in. (0.5 m.), 40 in. (1 m.) and 60 in. (1.5 m.) deep. Daily readings have been taken of the drainage which passes through the perforated plates which support the soil blocks, and since 1925 these daily readings have been supplemented by automatically recording apparatus installed by Dr Keen. The rain and drainage waters have been analysed for nitrate and chloride.

The monthly totals of rainfall and drainage have been published regularly in the *Annual Reports* of the Station. Gilbert (1891) reproduces these data up to 1891, Scott (1900) up to 1899, and Miller gives the yearly totals and monthly means to 1905.

The main conclusions reached by Lawes *et al.* from an examination of the records for the first ten years were:

- (1) There is no great difference between the records for the three gauges, and, consequently, no marked effect due to depth of soil. The 40 in. gauge may be slightly defective.
- (2) The mean annual drainage is about half the mean annual rainfall.
- (3) The annual drainage tends to be a greater fraction of the annual rainfall in years of high rainfall. Hence the annual drainage is more variable than the annual rainfall.
- (4) The annual deficit¹ differs little from year to year.
- (5) The deficit for the period October to March inclusive is generally less than one-third of the annual deficit, and agrees substantially with the evaporation from an open water surface (Greaves, 1876).

¹ The deficit for any period is the difference between the rainfall and the drainage. The precision with which the deficit is a measure of evaporation is discussed below.

(6) The deficit for the period April to September is less than the evaporation from an open-water surface, presumably because the soil surface is often dry.

(7) In January the mean monthly drainage is greatest in relation to the mean monthly rainfall; in July it is least.

(8) The drainage in any period must depend on the way in which the rain is distributed as well as on its total amount.

(9) The few cases where a month's drainage had exceeded its rainfall were due to snow or frost at the end of the previous month.

(10) All the chloride in the drainage water is brought into the soil by the rain. A considerable part of the nitrate is produced in the soil.

(11) The fluctuations in the chloride and nitrate concentrations indicate that the drainage takes place mainly through root-holes, worm-holes and fissures in the soil.

Since 1881 a number of additional observations and deductions have been recorded.

Warington (1900) noticed that the annual deficit had tended to decline. He thought this might be because stones had accumulated at the surface and were hindering evaporation. In a note on the review of the data by Scott, Gibbs (1904) suggested several possible causes: a decrease in the annual sunshine, an increase in the proportion of winter rain, a change in the physical state of the soil. The last possibility was further considered by Russell (1907).

Miller gave a table from which it appears that the annual deficit tends to be slightly greater in years of large rainfall, but he did not draw attention to this fact. He also pointed out that the 60 in. gauge tends to give slightly more drainage than the 20 in. gauge from January to May, and slightly less during the rest of the year, but offered no explanation.

To the monthly means of drainage (average for the three gauges) Crowther (1930) fitted a regression equation involving the mean monthly rainfall and the mean monthly air temperature and obtained significant correlations with both. He hoped this might approximate to a general expression for the dependence of drainage on rainfall and temperature.

Koshal (1934) made a statistical study in which the data for each gauge and each month were examined separately. He obtained thirty-six regression equations connecting the drainage in any one month with the rainfall and mean air temperature of that month. The regression coefficients on rainfall vary from nearly unity in January to about 0.5 in July. Thus an extra inch of rain falling in January produces almost an inch

more drainage, whereas an extra inch in July gives only about half an inch extra drainage.

To Koshal's surprise the regression coefficients on mean air temperature were small and mostly non-significant. Two, indeed, were positive, whereas he had expected that a rise in temperature would diminish drainage by increasing evaporation. His analysis left the greater part of the variation in drainage from month to month unexplained. He therefore suggested that an important part of this variation might be due to an annual cyclic change in the water content of the gauges.

Koshal obtained factors for the time variation of the constants in his equations but none was significant. Thus this very full statistical analysis failed to demonstrate any progressive change in the physical condition of the soil.

EXAMINATION OF THE AUTOMATIC RECORDS OF RAINFALL AND DRAINAGE

A. *Drainage after rain*

The drainage residue.

The automatic records show that drainage, when it occurs, continues after the rain which causes it has stopped. During these periods the drainage rate steadily decreases, becoming vanishingly small in 24 hr. in the case of the 20 in. gauge, and in about 48 hr. in the case of the 60 in. gauge. In the absence of further rain the amount of drainage still to come at any time in the drainage period we shall call the drainage residue.

Influence of temperature on drainage rate.

In a preliminary study of drainage after rain, measurements were made from the automatic charts of the drainage residue when the drainage rate had fallen to 0.0346 in. per hr. This rate corresponds to a 30° slope on the automatic charts and was selected because it is about the highest rate of drainage normally occurring after rain has stopped. The results for the 20 in. gauge shown in Table 1 cover all the cases from 1926 to 1939 in which rain stopped shortly before the drainage rate fell to 0.0346 in. per hr. and there was no more rain for 24 hr. In order to increase the number of observations, and thereby obtain better monthly averages, occasions were also included when additional rain not exceeding 0.05 in. fell soon after the 30° point. In these cases the drainage residue was obtained by subtracting the additional rain from the measured drainage.

Table 1 shows plainly that there is a seasonal variation of the drainage residue for a given drainage rate. Temperature must affect the rate of drainage through its influence on the viscosity of the percolating water. If, apart from the effect of temperature, the soil moisture conditions are always the same for the drainage residue, then the rate of drainage for a given drainage residue must be inversely proportional to the viscosity. Owing to the fortunate circumstance that the drainage curves on the charts are nearly exponential during the later stage of residual drainage, the drainage residues for a given drainage rate are almost inversely proportional to the drainage rates for a given drainage residue, i.e. the drainage residues (30° point) should be proportional to the viscosity.

Table 1. *Drainage residue beyond 30° point (20 in. gauge)*

Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
0.13	0.13	0.12	0.12	0.11	0.07	0.07	0.09	0.09	0.10	0.12	0.13
0.14	0.15	0.14	0.12	0.12	0.09	0.10	0.10	0.09	0.12	0.13	0.14
0.14	0.15	—	0.12	0.12	0.09	0.10	0.10	0.10	0.12	0.13	0.14
0.16	0.16	—	0.13	0.12	0.09	0.11	0.11	0.10	0.13	0.14	0.14
0.16	0.17	—	0.13	0.13	0.09	0.11	0.11	0.12	0.14	0.14	0.18
0.16	0.18	—	0.15	0.14	0.10	0.11	0.11	0.12	0.14	0.14	0.18
0.17	—	—	—	0.16	0.11	0.12	0.12	0.12	0.14	0.15	—
—	—	—	—	—	0.11	0.12	0.13	0.13	0.15	0.16	—
—	—	—	—	—	—	0.12	—	0.13	0.17	0.16	—
—	—	—	—	—	—	—	—	0.13	—	0.16	—
—	—	—	—	—	—	—	—	—	—	0.16	—
—	—	—	—	—	—	—	—	—	—	0.16	—
—	—	—	—	—	—	—	—	—	—	0.18	—
—	—	—	—	—	—	—	—	—	—	0.18	—
Mean 0.150	0.155	0.130	0.130	0.130	0.095	0.105	0.110	0.115	0.135	0.150	0.150

We have no measurements of temperature in the soil of the drain gauges but readings are taken daily (9 a.m.) at 4, 8, and 12 in. under bare soil nearby. The temperatures at 1 ft. were abstracted for each of the occasions and averages were taken by months. The corresponding average viscosity is plotted for each month on the same graph as the average drainage residues, the scales being adjusted so that the grand mean viscosity has the same ordinate as the grand mean drainage residue (Fig. 1).

Since soil temperature fluctuates daily and depends on depth, the 9 a.m. reading at 1 ft. is only a rough guide to the temperature of the water that percolated through the soil of the gauge. The variable time relationship will be largely smoothed out in the monthly averages and the errors so caused should be randomly distributed. There will also be systematic differences between the means of the observed soil temperatures and the proper mean temperature of the percolating water which may

itself have a seasonal variation. We, therefore, conclude that the phase and amplitude of the seasonal variation in drainage residue are largely accounted for by viscosity variations. The discrepancies of Fig. 1 are of a kind which suggest a slight change in physical structure between the dry months of spring and summer and the wet months of winter, but they are not large enough to indicate any appreciable increase in the water retaining capacity of the soil between these seasons.

It was thought that evaporation might check residual drainage. If this occurred there would be a tendency for residual drainage to be greatly dependent on the meteorological conditions during the first few hours after rain stopped. No such effect has been found, and we conclude that evaporation occurring after a fall of rain has no measurable effect on the drainage response to that rain.

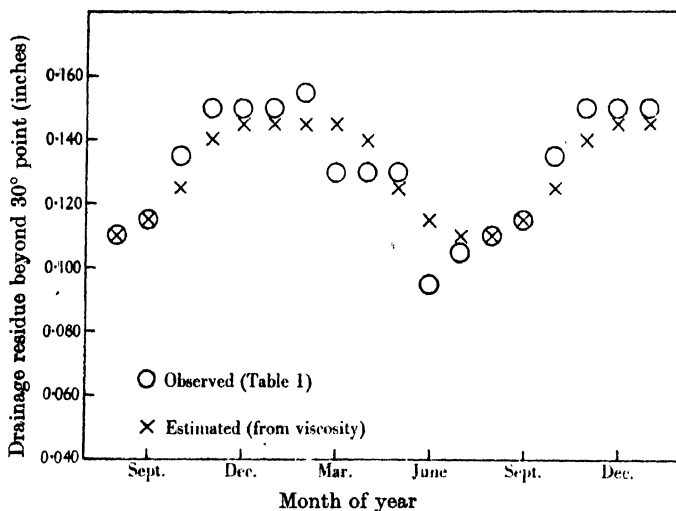


Fig. 1. Seasonal variation of drainage residue (20 in. gauge).

Maximum drainage rates.

There have only been a few occasions since 1925 where the automatic records show the attainment of a maximum rate of drainage. On 21 June 1936 0.90 in. of rain fell between 1.45 and 2.25 p.m. and a further 0.02 in. fell at 2.40 p.m. The 20 in. gauge was still draining at 5.00 p.m. when another violent storm broke. [The drainage rate had decreased to 0.0346 in. per hr. by 4.00 p.m.] From the June observations (Table 1 above) it follows that, had there been no more rain, only 0.095 in. more drainage would have been delivered after 4.00 p.m. This enabled us to find the drainage residue at times before 4.00 p.m.

In the case under consideration the drainage rate was at a maximum shortly before 2.25 p.m. when rain stopped. It seemed permissible during this period to obtain the drainage residue by subtracting the rain still to come. This procedure assumes that rain falling at this time added an equal amount to the drainage. The figures are given in Table 2 and the results are plotted in the top curve of Fig. 2.

Table 2. *Drainage rate and drainage residue (20) 21 June 1936*

Time p.m.	Rain to come (in.)	Drainage to come (in.)	Residue ($D - R$)	dD/dt (in. per hr.)
3.58	0.000	0.095 + 0.000*	0.095	0.035
47	0.000	+ 0.011	0.11	0.047
09	0.000	+ 0.050	0.15	0.095
2.50	0.000	+ 0.094	0.19	0.19
44	0.000	+ 0.113	0.21	0.26
34	0.020	+ 0.173	0.25	0.49
31	0.020	+ 0.202	0.28	0.70
28	0.025	+ 0.247	0.32	1.00
25	0.030	+ 0.303	0.37	1.00
21	0.085	+ 0.358	0.37	1.00
17	0.150	+ 0.416	0.36	1.00
14	0.230	+ 0.447	0.31	—
2.05	0.475	+ 0.453	0.07	—

* From Table 1.

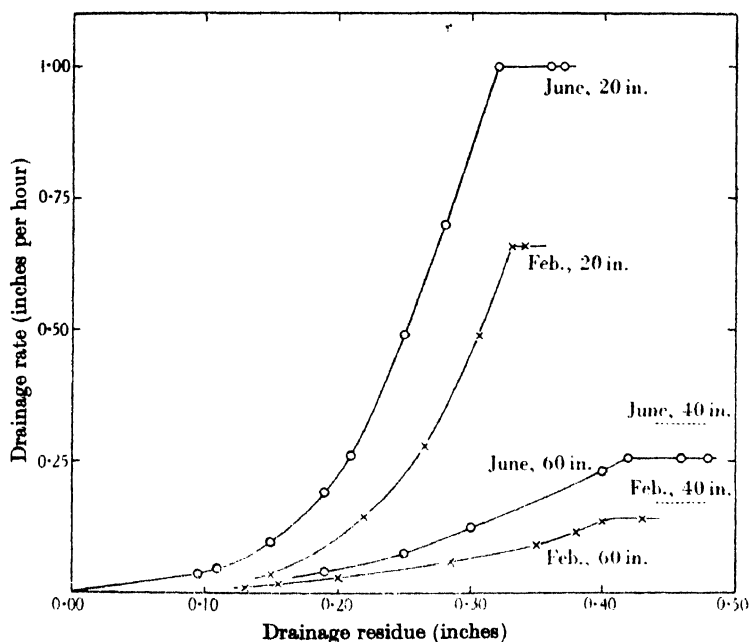


Fig. 2. Drainage residue and drainage rate.

It will be seen that the drainage rate was constant at 1 in. per hr. for drainage residues greater than 0.32 in. When the drainage residue exceeded this amount, the soil was evidently waterlogged, the amount in excess of 0.32 in. lying in puddles on the surface where it did not influence the drainage rate. In the case of the 60 in. gauge the maximum rate of 0.25 in. per hr. occurred for drainage residues greater than 0.43 in. The maximum rate for the 40 in. gauge was 0.32 in. per hr., but residual drainage from this gauge was augmented by a leak (p. 93) and so was not determined.

The downpour which occurred later in the same day precipitated 1.5 in. between 5.30 and 5.50 p.m. We have estimated that for some minutes the water was 1 in. deep on the soil of the gauges. During this time the drainage rate was 1.44 in. per hr. in the case of the 20 in. and 0.58 in. per hr. in the case of the 40 in. gauge. The pen of the 60 in. gauge failed to mark. These rates are appreciably higher than the maximum rates in Fig. 2. The probable explanation is that water deep enough to form a continuous sheet can flow sideways and feed any place where drainage is particularly easy. The periphery of the gauge where the soil block lies against the retaining wall provides a likely channel for the extra rapid drainage. Unfortunately the automatic equipment cannot record more than 1 in. of drainage in a day. Consequently the drainage residues after this flooding are not obtainable.

The other two curves in Fig. 2 were obtained from the records for 25 February 1937. The maximum drainage residues were only 0.34 and c. 0.42 in. for the 20 in. and 60 in. gauges respectively and the flat parts of the curve are very short. Support for the conclusion that on this occasion also the soil was waterlogged is obtained from inspection of the charts. During the period when the drainage curve was straight a small amount of rain fell at a nearly infinite rate and had no effect on the drainage slope.

On about a dozen occasions the drainage rate for the 20 in. gauge has exceeded 0.3 in. per hr. the maximum drainage residues ranging from 0.22 to 0.32 in. The 20 in. rate reached 0.82 in. per hr. on 9 October 1935 (i.e. midway between the June and February maxima), but owing to an uncertainty in the synchronization of the rainfall and drainage charts the value of the corresponding drainage residue can only be given as between 0.28 and 0.34 in.

The data for maximum drainage rates are summarized in Table 3. The first column under each date shows the maximum rates; these fall off with increasing depth in the same way for each date. The second

column gives a measure of the resistance to water movement; this is corrected for viscosity differences in the third column. This correction assumes that the viscosities at all depths are determined by the mean soil temperature (at 4, 8 and 12 in.) on the morning of the rainfall. For the shallowest gauge this is probably of the correct order, but may be considerably in error for the others. For instance; if the gauges were not partly isolated from heat movements from below one would expect the June and October temperatures to be nearly equal at 40 in., i.e. the best comparison between June and October for the deeper gauges is between the entries in column two. In the same way the February resistances are probably under-estimated. The fourth columns give the resistances of the lower two 20 in. of soil and in all three cases these are nearly equal and about five times as great as those of the top 20 in.

Table 3. *Seasonal change in maximum drainage rate*

Depth of gauge in.	June 1936					Feb. 1937					Oct. 1935				
	Max. drainage rate in. per hr.	Depth ÷ max. drain. rate = res.	Res. ÷ viscosity (June $\eta = 1$)	Δ (res./ η)		Max. drainage rate in. per hr.	Depth ÷ max. drain. rate = res.	Res. ÷ viscosity	Δ (res./ η)		Max. drainage rate in. per hr.	Depth ÷ max. drain. rate = res.	Res. ÷ viscosity	Δ (res./ η)	
20	1.00	20	20			0.66	30	20			0.82	25	21		
40	0.32	125	125	105		0.17	235	155	135		0.32	125	108	87	
60	0.25	240	240	115		0.14	428	282	127		0.26	231	201	93	

It appears then, that the gauges have a system of comparatively wide drainage channels which may extend down to about 20 in.; below this depth the structure is nearly uniform, the drainage channels being narrower and/or fewer. Fig. 1 and Table 3 suggest that there is a slight change in water-holding capacity between June and February. Thus from Table 3 the ratio of the February and June resistances for unit viscosity below 20 in. is 1.19, and as the resistance is proportional to the inverse cube of the channel width, the change in the latter is about 6 %. The corresponding value for the February-October change is about 15 %. The changes in the air content at field capacity will thus be of the same order and the next section will show that these changes in a small quantity are quite insignificant fractions of the water content at field capacity.

The air content of the drained soil.

From Fig. 2 it is quite clear that starting from a waterlogged state, but with no water lying on the surface and no more rain falling, the 20 in.

gauge would only discharge about 0.32 in. When the soil is waterlogged practically all the air is driven from its pore space. Hence when drainage stops and no water has been lost by evaporation only $0.32 \times 100/20 = 1.6\%$ of the volume of the 20 in. soil block is occupied by air. Although this may seem a small value for the air content of fully drained soil, it is quite consistent with the volume, weight and water content of a series of soil samples taken from fallow land close to the gauges in 1870.

The sampling tool was an iron frame 9 in. deep and 6 in. square. It was driven in till its upper edge was flush with the surface of the ground, and the soil so enclosed was dug out to 9 in. and immediately weighed. The surrounding soil was then removed, the frame was driven down another 9 in. and a second sample was dug out and weighed. The process was repeated till six samples had been taken. The samples were oven dried and reweighed in the laboratory.

The intention when the samples were taken was to find the moisture content of the soil, and only the percentages of water in the samples (excluding stones $> \frac{1}{4}$ in.) were published at the time (Lawes & Gilbert, 1871). The experimental figures have been preserved and are given in

Table 4. *Samples taken from fallow soil close to the drain gauge in 1870*

9 in. section	Sample as taken (stones included) oz.	Sample oven dried (stones included) oz.	Water by difference oz.	Vol. of solids ($\rho = 2.7$) fluid oz.	Vol. of solids plus water fluid oz.	Nominal vol. of sample fluid oz.
1	339	291	48	112*	160	187.5
2	339	251	88	93	181	187.5
3	341	238	103	88	191	187.5
4	348	252	96	93	189	187.5
5	354	256	98	95	193	187.5
6	328	236	92	88	180	187.5

* Specific gravity of solids in first section taken as 2.6.

Table 4. The second column shows the original wet weight (stones included), the third the dry weight (stones included) and the fourth the water content of the samples in ounces. Assuming that the solids have a mean specific gravity of 2.7, the fifth column gives the volumes occupied by the solids in fluid ounces. The sixth column gives the volume occupied by solids and water in fluid ounces.

The volume of each sample should have been $3/16 = 0.1875$ cu. ft. = 187.5 fluid oz. It will be seen that in three cases the combined volume of solids and water exceeds 187.5 fluid oz. Owing to the difficulty of driving the frame down precisely 9 in. each time, the total volume of each

sample evidently was not exactly 187.5 fluid oz. Consequently we cannot compute the air content precisely; but it is evident that below 9 in. it is quite small. The higher air content of the top 9 in. must be attributed partly to loss of water by evaporation and partly due to loose structure produced by cultivation. The top soil of the drain gauges has not been cultivated since 1870, and the estimated air contents of 1.6 % in the top 20 in. of soil and of 0.7 % in the top 60 in. (i.e. 0.25 % in bottom 40 in. of deepest gauge) are not inconsistent with the sampling figures.

B. Drainage during rain

The first response of the gauges occurs some time after the onset of rain, the delay being ascribable to two causes. The first is the need to bring the soil moisture up to field capacity; if the quantity of rain falling

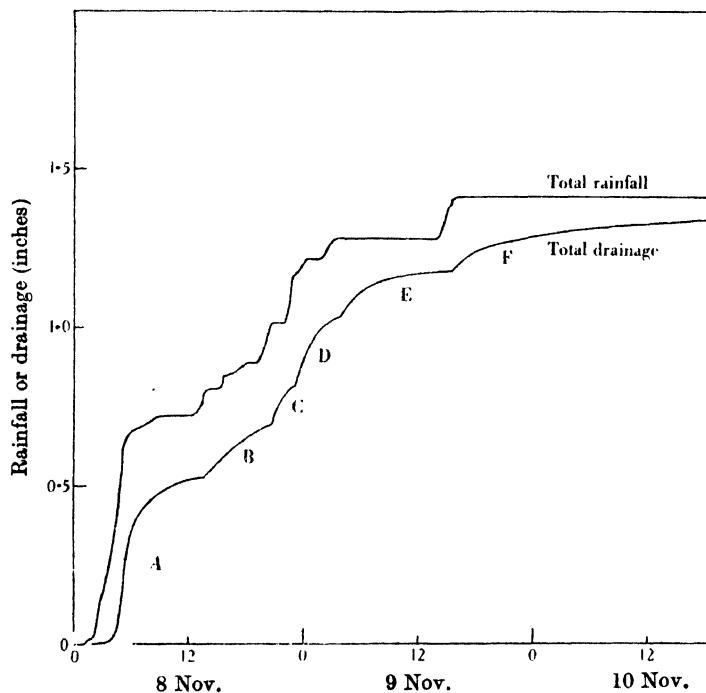


Fig. 3. 20 in. drainage curve (8-10 Nov. 1926).

is not sufficient to do this, then, with occasional exceptions to be noted below, there will be no drainage. The second is due to the finite time required for the water to move down from the surface to contribute to the head causing drainage. Assuming that the rate of rainfall is steady,

the drainage head increases steadily, and with it the drainage rate, until the latter is equal to the rainfall rate. A change in rainfall rate tends to produce a corresponding change in drainage rate but because of the time lag in moving through the gauge the adjustment to new conditions is not instantaneous and the drainage curve thus tends to give a smoothed out picture of the rainfall curve at an earlier epoch. The curves for November 1926 (Fig. 3) show this and also the time lag in the response to new falls

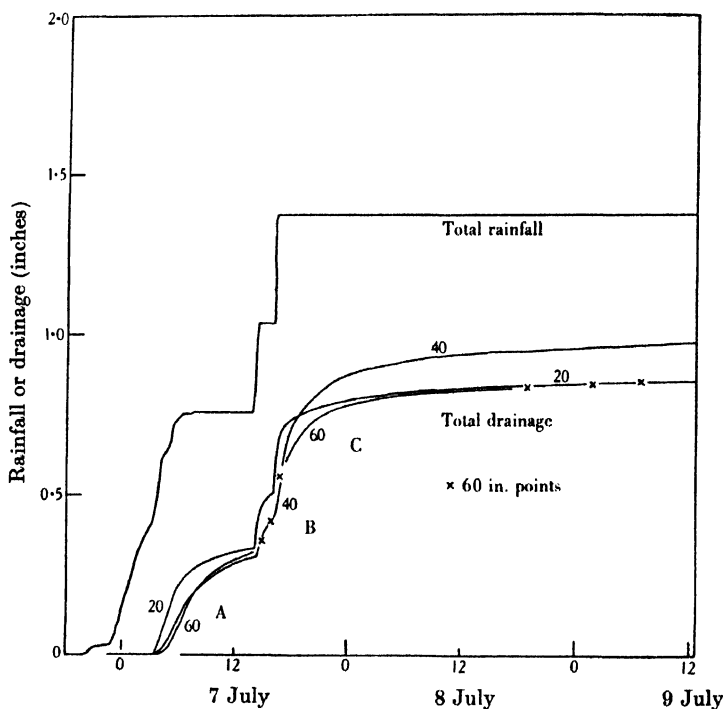


Fig. 4. 20, 40 and 60 in. drainage curves (6–9 July 1927).

of rain. The effect of previous evaporation on the response is shown in the lower part of Fig. 4, where about 0.45 in. of rain fell before drainage began. The total rain in the first fall was 0.76 in. and from the 30° point and Table 1 the drainage would have been 0.35 in., i.e. 0.41 in. were needed to bring the soil moisture up to field capacity. This is less than the amount which had fallen when drainage began, the difference being a measure of the lag in response of the gauge, and this is the general rule for the behaviour of the gauge. There are exceptions, one of which is illustrated in Fig. 5, imperfectly because of the reduced scale, and details quoted below are taken from the 20 in. charts. The rain on 13 August

1937 ended a warm dry period of 3 weeks. In the first shower 0.17 in. fell without effecting drainage; when the second heavy fall occurred 8 hr. later, most of the first would probably have re-evaporated; because of this uncertainty, two figures will be given for rainfall: R and $(R + 0.17)$. The drainage response to the two later showers gave curves which were identical beyond the 30° point and the second was used to estimate the drainage residue of the first. Drainage began at 5.45. Previous rain was

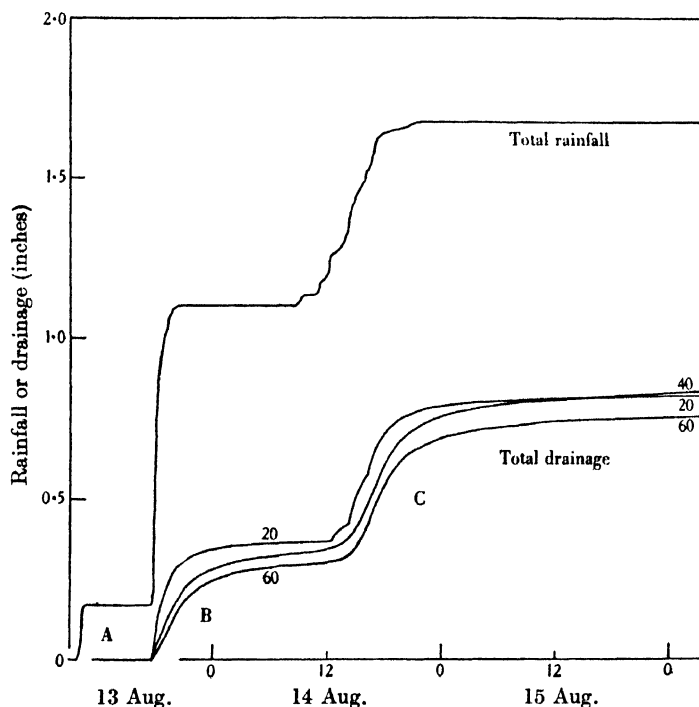


Fig. 5. 20, 40 and 60 in. drainage curves (13–15 Aug. 1937).

0.105 (0.275) in. The total drainage was 0.385 in., the total rain was 0.935 (1.105) in., i.e. the deficit was 0.550 (0.720) in. If the rain had fallen slowly, gradually bringing up the gauge to field capacity, an amount at least equal to the deficit would have been needed to start drainage; the observed value is very much less, suggesting that water passed through the gauge without first saturating the soil. Part of this would pass down the gap between the soil and its retaining wall, and part through the body of the soil, where, because of the very rapid rainfall rate, air in the pores would be unable to escape, thus making these pores inaccessible to water which would then appear as abnormal drainage. The effect is

one which is to be anticipated whenever rain starts and is maintained at a rapid rate of fall on soil which contains air. Returning to Fig. 4, we have a further example in the response to the later rains on 7 July. The first of these two heavy showers occurred after air had had an opportunity of entering the soil; it amounted to 0.28 in. and was followed about 2 hr. later by a fall of 0.34 in. We have estimated the drainage responses (from 30° points) as 0.24 in. for each fall, i.e. the deficits are 0.04 and 0.10 in. respectively. The evaporation opportunities for producing these deficits were 7 hr. mostly sunny, about midday, and 2 hr. about midnight; hence one would anticipate a comparatively large deficit for the first and a very small one, probably zero, for the second. We conclude that air was entrapped during the earlier of these two heavy falls; there is no evidence to show that the same was not true of the second.

There may be abnormal drainage in winter. During falls of snow the gauges may receive more or less than the average amount because of drifting. If the ground is frozen before snow falls, the eventual drainage will depend upon the rate at which the soil thaws as well as that at which the snow melts. Snow fell in January 1940 after a long period of frost in which the soil froze down to a foot or more. During the eventual thaw the 60 in. gauge was first to begin running, then 40 in. a day later and finally 20 in., a further day later, suggesting that the shallowest gauge had been frozen from the bottom as well as from the top. Even without snow, the effect of frost is important. It has a drying action and in addition to immobilizing water which would otherwise drain away, it empties some of the smaller pores which would not normally be emptied by free drainage. Thus frost during a drainage period will hold water back and whilst the ice is still present further rain will have this extra deficit to make up. When the ice thaws, the amount frozen will, of course, be added on to the normal drainage. The first and third of these phases can be seen in Fig. 3. The grass minimum on 8 November was 26° F.; on the 9th, 35° F. The deficit of the first shower is obviously greater than the final deficit and drainage was presumably held up by freezing, the ice melting slowly until the penultimate shower of 9 November, when the deficit became appreciably less.

The information from the 20 in. curves of Figs. 3-5 is summarized in Table 5.

Between periods B and C of November 1926 there is a slight increase in the accumulated deficit which may be due to the drying action of the frost, but as the magnitude of the change is close to the inaccuracy of extrapolation, the evidence is not conclusive.

Table 5. *Effect of soil conditions on drainage*

Date	Drainage period	Rain	Estimated drainage	Rain when drain ran	Deficit	Accumulated deficit
Nov. 1926	A	0.72	0.60	0.17	0.12	0.12
	B	0.17	0.18	0.07	-0.01	0.11
	C	0.125	0.10	0.12	0.025	0.135
	D	0.205	0.21	0.15	-0.005	0.130
	E	0.060	0.11	0.06	-0.05	0.080
	F	0.130	0.15	0.12	-0.02	0.060
July 1927	A	0.76	0.35	0.45	0.41	0.41
	B	0.28	0.24	?*	0.04	0.45
	C	0.34	0.24	?	0.10	0.55
Aug. 1937	A	0.17	0.00	—	0.17 +	0.17
	B	0.93	0.38	0.10	0.55	0.72
	C	0.58	0.44	0.16	0.14	0.86

* Uncertain because of stretching of recording paper in humid atmosphere; this is unimportant in November 1926, and allowed for in all charts after 1934 and thus in August 1937.

C. *Natural periods*

The small and constant values of the monthly drainage residues (Table 1), obtained for widely differing drying conditions at the surface, suggest that when evaporation begins at the surface the moisture distribution in the gauge is such that upward movement is confined to the surface inch or two and drying at the surface during the period of drainage does not affect the total discharge. Assuming that, in general, drainage only occurs when the water content exceeds field capacity, the moisture changes between the ends of two falls of rain causing drainage can be written

$$(FC + DR_1) - DR_1 - E_{12} + R_2 = FC + (D_2 - DR_2) + DR_2.$$

Otherwise: at the time the rain stops the gauge contains its field capacity (FC) and a drainage residue (DR_1). Losses of two kinds occur: (1) the drainage residue is gradually discharged, and (2) evaporation (E_{12}) occurs from the surface. Rain (R_2) falls, and after bringing the gauge to field capacity, a certain amount drains during the rain period ($D_2 - DR_2$), leaving, at the end of the fall, a residue DR_2 , which must of course make the total drainage equal to D_2 . The equation reduces to

$$E_{12} = R_2 - D_2.$$

If R_2 falls in one shower, E_{12} is the total amount lost by evaporation. If between R_1 and R_2 there is a number of small falls which do not produce any drainage, then E_{12} is the net loss by evaporation: the total evaporation over the period is

$$\Sigma E_{12} = \Sigma R - D_2,$$

where ΣR includes R_2 . Obviously we may add the evaporations for a number of such periods to obtain

$$\Sigma E = \Sigma R - \Sigma D, \text{ i.e. evaporation} = \text{deficit (p. 1),}$$

where the following conditions must be noted:

(1) ΣR is measured from the end of one fall of rain causing drainage to the end of another also causing drainage.

(2) ΣD is measured between the cessations of the drainage responses to these falls of rain, i.e. *the drainage period is not in phase with the rainfall period.*

Periods which satisfy these conditions we shall call "natural periods". They may be as short as a few days; they may extend over several months. For certain arbitrary periods the error in assuming that they are natural periods is small. Thus for a year (1 January–31 December) the phase difference is unimportant and as December and January are normally months of heavy rainfall, the showers of which rarely fail to cause drainage, the evaporation attributed to 365 (6) days ($\Sigma R_y - \Sigma D_y$) may actually be due to a few days more or less—a negligible error. Similarly, periods of six calendar months which begin and end in seasons of comparable weather may be treated as natural periods. Thus in the later general discussion we shall consider April to September, and October to March as approximating closely to natural periods, but one unsatisfactory aspect of a division between September and October will shortly appear. Individual calendar months will rarely be natural periods. The phase difference may be important in cases where rain falls on the last day of the month; in winter months, for instance, neglecting it may lead to an estimate of drainage which exceeds the month's rainfall. More generally, the beginning of the natural period, which is the end of the last fall of rain causing drainage preceding the beginning of the month, may be several days, or in a dry summer perhaps several weeks earlier than the first day of the calendar month, so, assuming that the end of the natural period is the end of the calendar month, the deficit ascribed to the calendar month will actually be due to the evaporation over a much longer period. Similarly, assuming the beginning of the calendar month to be the beginning of a natural period, the latter may only last for a few days, the rest of the month being rainless, or, less seriously, 'drainless'. In this case the estimated deficit for the month will be due to evaporation over a shorter period.

Another trouble arises when the deficits are correlated with meteorological conditions. For instance; an October deficit may be due to a

period including two weeks of September. Ascribed to the 31 days of October it will be an over-estimate and the correlation will be further vitiated by the assumption that the deficit is due entirely to the meteorological conditions during these 31 days.

Over a long period of years, and where there are no violent changes of weather from one month to another, it is probable that errors will cancel out. Thus the aggregate deficit for 50 Augusts probably corresponds to the evaporation of 50 months having the mean meteorological conditions of 50 Augusts. October is a likely exception. September is comparatively warm and dry; October is colder and is the wettest month of the year. It is very probable that the October deficit often includes a contribution from dry days at the end of September which is not compensated by a corresponding loss from October to November. Thus for October we may expect

$$\Sigma R_0 - \Sigma D_0 > \Sigma E_0.$$

These approximations to natural periods will be used in the later general discussion and also in a short note by Mr Sahni which follows this account. He considers periods of 2-13 days, using the daily totals of rainfall and drainage. In practice one cannot always be certain of the last rain to cause drainage; a heavy fall causing drainage may be followed 12 or 24 hr. later by a light shower whose effect on the drainage cannot be established with certainty. Thus for a rapid survey from the daily totals it is more convenient to take the period as between the last days of drainage, or between very low drainage minima (< 0.005 in. per day). The error may be of the order of one-half to one day, i.e. of the same order as that which would be present in any case in using daily totals. From this it would seem that no period shorter than 5 days should be considered, but as Sahni's data will show, it is possible to work with 2-day periods which, with one exception, show no anomalous behaviour.

DAILY TOTALS OF RAINFALL AND DRAINAGE

Drying out of 20 in. gauge

In the preceding section we saw that the deficit for a given fall is a measure of the net water lost by evaporation in a previous period of drying. From earlier sections and Figs. 3-5 it will be apparent that, except for rainfalls near the end of a meteorological day, the greater part of the drainage is complete on the day of the rain, and to a very good

approximation the difference between the daily totals may be taken as giving the deficit.

The values of the deficit so obtained will be over-estimates of the evaporation, but they can be very quickly read off from the daily totals to give the order of magnitude of the water loss from fallow soil during dry seasons. Table 6 (a) shows all the occasions between 1925 and June 1936 when this water loss exceeded about 0.40 in. The second part (b) of the table is a more detailed record of the responses to heavy rain in all seasons for 1936-9 in which the values of D have been estimated from the charts, and the amounts of rain required to start drainage have been included. Comparing these with the values of $R - D$, they appear to be generally of the same order. There are a few cases in which the rain needed to start drainage is appreciably less than $R - D$ and these probably arise from the entrapping of air by rapidly falling rain.

The first part of the table shows that in normal summers the net water loss rarely exceeds 0.7 in., this being the amount to bring the soil to field capacity. We have already seen that to waterlog the soil about 0.3 in. of water is needed in excess of field capacity (p. 82) so that in the dried-out state of a normal summer only 1 in. of water would be needed to replace the total air content of the soil of the 20 in. gauge, i.e. the air content of this fallow undisturbed soil rarely exceeds 5 % of the total volume. The effect of cultivation (again without crop) appears

Table 6. *Response of 20 in. gauge to rain*

(a) 1925-36

Date	R	D_{20}	$R - D_{20}$	Date	R	D_{20}	$R - D_{20}$
20. vii. 25	0.85	—	0.85	2. iv. 31	0.44	—	0.44
22. vii. 25	1.10	0.67	0.43	5. vi. 31	0.41	—	0.41
23. viii. 25	1.37	0.82	0.45	14. vii. 31	1.18	0.47	0.71
2. vi. 26	0.64	0.23	0.41	2. ix. 31	0.47	0.07	0.40
4. vii. 26	0.45	—	0.45	21. v. 32	0.72	0.30	0.42
19. vii. 26	0.45	0.02	0.43	30. vi. 32	0.68	—	0.68
18. vi. 27	0.39	—	0.39	11. vii. 32	0.49	0.01	0.48
22. vi. 27	0.46	—	0.46	11. viii. 32	0.69	0.08	0.61
6. vii. 27	0.77	0.30	0.47	20. viii. 32	0.69	0.04	0.65
11. vii. 27	0.52	0.07	0.45	22. ix. 32	0.50	0.09	0.41
7. viii. 27	0.70	0.08	0.62	12. ix. 33	0.53	—	0.53
9. ix. 27	0.78	0.16	0.62	25. vi. 34	0.76	—	0.76
27. vii. 28	0.84	0.10	0.74	28. viii. 34	0.75	—	0.75
12. iv. 29	0.43	0.02	0.41	15. ix. 34	0.98	0.32	0.66
24. v. 29	1.04	0.40	0.64	18. v. 35	0.42	—	0.42
1. x. 29	0.68	—	0.68	30. v. 35	0.42	—	0.42
5. x. 29	0.67	0.01	0.66	18. vii. 35	0.64	—	0.64
20. x. 29	0.72	0.32	0.40	30. viii. 35	0.64	—	0.64
3. iv. 30	0.50	0.04	0.46	12. vi. 36	0.48	—	0.48
25. v. 30	0.51	0.13	0.38	21. vi. 36	3.20?	2.62?	0.58?
18. vi. 30	0.72	0.06	0.66				
20. viii. 30	0.42	—	0.42				
13. ix. 30	0.40	0.01	0.39				

Table 6 (cont.)

(b) 1936-9

Date	R	D	R - D	Rain when drain ran	Previous rain
1936					in.
2. vi.	0.48	—	0.48	—	0.53 in preceding month
12. vi.	{0.54 0.33	— 0.15	0.54 0.18	{— 0.15}	0.80 in preceding 12 days
21. vi.	{0.92? 1.87?	? ?	? ?	{0.47 0.12}	0.61 in preceding 7 days
2. vii.	0.49	0.22	0.27	>0.25	0.66 in preceding 7 days
7. vii.	0.75	0.34	0.41	0.24	0.49 5 days before
10. vii.	0.47	0.15	0.32	0.31	0.75 3 days before
15. vii.	0.70	0.43	0.27	0.39	0.33 2 days before
20. ix.	1.18	0.75	0.43	0.42	{0.18 in preceding week 1.50 in preceding 3 weeks
31. x.	0.64	0.54	0.10	0.18	0.95 in preceding 3 weeks
11. xi.	0.96 + ?	?	?	0.21	1.13 in preceding 10 days
1937					
16. iv.	1.01	0.90	0.11	0.22	0.92 in preceding 10 days
11. v.	0.68	0.39	0.29	0.16	0.14 5 days before
20. v.	0.59	0.32	0.27	0.36	0.18 2 days before
23. v.	0.50	0.35	0.15	0.30	0.59 3 days before
13. viii.	{0.26 0.94	— 0.36	0.26 0.58	{— 0.08}	0.00 in preceding 3 weeks
14. viii.	0.58	0.45	0.13	0.15	1.20 on previous day
15. ix.	0.49	0.01	0.48	0.15	0.45 in preceding 4 weeks
17. ix.	{0.33 0.46	0.21 0.46	0.12 0.00}	0.08	0.26 on previous day
1938					
15. i.	0.49	0.49	0.00	0.08	0.25 on previous day
12. viii.	0.79	0.25	0.54	0.56	0.68 in preceding week
28. viii.	0.45	—	0.45	—	0.43 in preceding 16 days
18. xi.	0.45	0.30	0.15	0.20	0.34 in preceding week
25. xi.	0.63	0.58	0.05	0.16	0.99 in preceding week
27. xi.	0.40	0.38	0.02	0.14	0.63 2 days before
9. xii.	0.82	0.71 app.	0.11 app.	0.24	0.20 in preceding 5 days
1939					
29. iv.-1. v.	1.55	0.92	0.63	0.56	0.47 in preceding 3 weeks
15. v.	0.57	0.24	0.33	0.35	0.33 on previous day (no D)
10. vi.	0.55	—	0.55	—	0.00 in preceding 3 weeks
15-16. vi.	0.30 + 0.23	0.00 + 0.12	0.41	0.53	0.37 in preceding 4 days
2-3. viii.	0.40 + 0.39	0.00 + 0.27	0.52	0.55	0.35 in preceding 11 days
20-21. viii.	0.24 + 0.40	0.00 + 0.17	0.47	0.56	0.00 in preceding 9 days
1. ix.	0.55	0.11	0.44	0.52	0.00 in preceding 5 days
2. ix.	0.32	0.22	0.10	0.29	0.55 on previous day
8-9. x.	0.50 + 0.24	0.39	0.35	0.39	0.40 4 days previous (no D)
13-15. x.	1.69	1.46	0.23	0.21	0.06 in preceding 3 days
22-27. xi.	1.69	1.57	0.12	0.12	0.01 in preceding 3 days

in the data for the top 9 in. for midsummer 1870 (Table 4). The air content is about 15 % in this layer, and 8 % for the top 20 in., showing an appreciable increase.

The second part suggests that the greater part of the loss occurs in the first few days after rain. Thus the deficits after 5 days were 0.4 and

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0.45 in. (7 July 1936 and 11 July 1927); after 9 days, 0.65 in. (20 August 1932); and after 3 weeks' drought, only 0.84 in. (13 August 1937). An extreme case occurred in 1921, when after a severe drought interrupted by nominal rainfall in June, July and August, a fall of 2.0 in. was recorded early in September, the responses being:

$$\begin{aligned} D_{20} &= 0.70, & R - D_{20} &= 1.30, \\ D_{40} &= 0.63, & R - D_{40} &= 1.37, \\ D_{60} &= 0.59, & R - D_{60} &= 1.41. \end{aligned}$$

Incidentally the response of the gauges is in order of depth. The log book of the gauges records the presence of cracks in the soil at the beginning of the fall, and the first part of the drainage would certainly be expedited by these cracks, but by the end of the drainage period the soil would be saturated and we may take the above figures as a measure of the extent of the drying out under extreme conditions. These examples illustrate what is apparent from the complete records, that the initial rate of drying is high (while the surface is wet) but becomes very low when the top layer of soil has dried. We should thus expect more evaporation when a given amount of rain is spread over a number of showers, especially in summer.

Effect of rainfall distribution on drainage

In Table 7 are shown the effects of a difference in rainfall distribution in summer and in winter. These are taken from the daily totals and show (a) the response to heavy falls concentrated in one day, and (b) similar total falls spread over a month, (i) in winter, and (ii) in summer. Heavy falls in a short time produce an appreciable amount of drainage, whatever the season, but if the same amount falls in a series of small isolated showers there may be no drainage during summer months whereas the winter drainage is not appreciably reduced.

We may, therefore, expect a regression equation connecting drainage and rainfall to be more accurate for winter months than for summer months.

Table 7. *Effect of distribution on response of 20 in. gauge*

(a) Heavy falls (>1.00 in.) in a day

Date	R	D
22 July 1925	1.100	0.673
23 Aug. 1925	1.370	0.822
28 Jan. 1927	1.070	0.896
14 Sept. 1927	1.703	1.516
28-29 Nov. 1927	1.318	1.288
24 May 1929	1.040	0.405
14 July 1931	1.182	0.468
21 June 1936	3.200?	2.618?

Table 7 (*cont.*)

(b) Similar falls spread over a month

(i) Winter			(ii) Summer		
Month	<i>R</i>	<i>D</i>	Month	<i>R</i>	<i>D</i>
Feb. 1929	0.789	0.708	June 1929	1.023	0.002
Feb. 1930	0.855	0.612	July 1929	1.417	0.001
Mar. 1930	1.451	0.712	June 1931	1.520	0.007
Jan. 1931	1.704	1.231	June 1932	0.850	0.006
Dec. 1931	1.109	0.643	June 1933	1.033	0.000
Dec. 1932	0.733	0.453	July 1933	1.425	0.000
Nov. 1933	1.471	0.890	June 1934	1.750	0.000
Jan. 1935	1.072	0.692	July 1934	1.130	0.000
Mar. 1936	1.413	0.437	July 1935	0.961	0.000
			Aug. 1935	1.635	0.000
			July 1937	1.779	0.000

Comparison of the gauges

If the three gauges differed only in depth one would expect certain regular differences in the responses to rain. These would be most marked in periods of intensive drying when the gauges might be expected to respond in the order $D_{20} > D_{40} > D_{60}$. The annual totals show that D_{40} has, with few exceptions, been greater than either D_{20} or D_{60} , the difference between these being small and of variable sign. Various explanations of this departure from expectation have been offered, including differential washing down of silt, differences in physical structure, and a leak in the 40 in. gauge casing which Lawes & Gilbert re-cemented in 1874.

Examination of the 1921 drought data (above) shows that the responses were in the expected order. This is usually true after dry periods when the automatic charts indicate that the first response is $D_{20} > D_{40} > D_{60}$. After a further fall the order gradually changes to $D_{40} > D_{20} > D_{60}$, and still more rain leads to $D_{40} > D_{60} > D_{20}$ (see Figs. 4, 5). This suggests that there is a leakage through the cement casing of the 40 in. gauge, and perhaps of the 60 in. gauge also, that occurs when the outer soil is very wet. Confirmation of this suggestion has been made in two ways.

Miller gives figures for the chloride content of rain and drainage water for the period 1877-1904. The annual means are:

Rain	15.15 lb. per acre,
20 in. gauge:	14.84 lb. per acre,
40 in. gauge:	15.89 lb. per acre,
60 in. gauge:	14.64 lb. per acre.

The corresponding data obtained by Miller up to 1915 (reviewed by Russell & Richards 1920) show similar agreements and differences and

it is obvious that more chloride is coming through the 40 in. gauge than is falling on the surface in the rain and we must conclude that the extra amount is being drawn from soil outside the gauge, i.e. there is a leak into the 40 in. gauge. The symptoms are compatible with a crack fairly near the surface which is only effective when rainfall has been sufficient to saturate the grass-covered surround, i.e. when the fall has made up the deficit due to combined evaporation and transpiration of the grass.

A direct test was made in June 1939, when, after a dry May, the ground round the gauges had shrunk away from the walls. Water was run into the fissures and after 18 hr. the gauges recorded:

$$D_{20} \text{ 0.000; } D_{40} \text{ 0.020; } D_{60} \text{ 0.007.}$$

Both chloride and direct test lead to the conclusion that the 40 in. records have been affected by leakage. The evidence is not so conclusive for the 60 in. gauge. Comparison of the monthly values of D_{20} and D_{60} , shows that the former is usually greater in autumn and early winter, but from January into the spring the reverse is the case. The first effect is presumably due to the deeper drying but this is masked in the wet winter months by slight leakage. After dry summers D_{20} may be persistently greater than D_{60} for every month from September to the following February (e.g. 1914, 1921) whereas after wet summers the change-over occurs in October-November. These effects can be partly accounted for by a leak in the deeper gauge, and while the existence of a leak is non-proven, the fact that there is reasonable doubt about the water tightness of the walls is held to be sufficient reason for leaving consideration of the 60 in. records out of the later general discussion.

PUSA DRAIN-GAUGE DATA

At this stage it will be useful to consider how a difference in soil and weather conditions affects the drainage response. Leather (1911) gives daily rainfall and drainage totals for gauges at Pusa. The drain gauges are 1/1000 acre in area, two being 6 ft. deep and two 3 ft., one in each pair being cropped and the other left fallow. The soil is alluvial with 40 % of fine chalk, the first 2 ft. being loam, the next two more sandy and the bottom 2 ft. are stiff clay. Run-off is measured when standing water would exceed 2 in. The data cover the years 1906-10 but our discussion will be confined chiefly to those for 1909 in which rain was heavy and drainage considerable. Leather's main conclusion was that water passed very uniformly through the soil and not chiefly by means

of large channels. The daily records indicate that the gauges run for 5 or 6 days after rain, about twice as long as the Rothamsted gauges. From the nature of the soil, one would expect the Pusa time to be shorter, assuming equal degrees of homogeneity, and the fact that the Rothamsted gauges discharge completely in a shorter time seems to indicate that they have some coarse macrostructure which facilitates percolation. The Pusa records show that where root holes are present they assist drainage. (a) The cropped gauges respond to rain about one-half to one day earlier than the fallow gauges. (b) The maximum daily amounts of drainage are larger for the cropped than for the fallow gauges. On three occasions the cropped gauges exceeded 3 in. per day, and exceeded 2 in. seven times. The fallow gauges never exceeded 3 in. in a day and only twice exceeded 2 in. (c) Where run-off has been recorded the amounts are greater from the fallow gauges. Summarizing, the cropped gauges appear to be more permeable so that (a) they respond more rapidly to rain, (b) larger amounts can pass through quickly, and (c) surface accumulation is less.

An estimate of the extent to which the soil had dried cannot be made as precisely as was possible with the Rothamsted data because of the longer time lag between the incidence of rain and onset of drainage, the longer period for which the drains run, and the high rate of evaporation while the surface is wet. Making allowances for these factors so that the figures for the drying out is an over-estimate, it appears that at the beginning of the 1909 monsoon, after 21 months without drainage, it was about $12\frac{1}{2}$ in. for the deep gauges and 10 in. for the shallow ones. The drying out before the monsoon of the following year was much less, being about 3 or 4 in. This is probably a more normal value than the other, because the effect of 21 months drying had probably extended so far down the gauge that convection of air through the soil could take place easily, and the drying rate would be accelerated thereby. This type of drying would not occur in a field soil, and it is possible that 10 in. is a considerable over-estimate of the loss from field soils even in abnormal years.

GENERAL DISCUSSION OF SEASONAL EVAPORATION

This discussion is primarily an application of the conception of natural periods to the detailed analysis of Koshal. He obtained partial regression equations representing the drainage to be expected in any month in terms of the mean air temperature and rainfall of the month, with allowance for secular changes in these variables. Postponing considera-

tion of the secular changes, we have to consider a series of equations of which the following, for January, is typical:

$$D_{20} = 1.9043 + (0.96729 \pm 0.03848) R' + (0.00232 \pm 0.01294) T',$$

where R' and T' are deviations from the monthly means. The equations can be rearranged to give expressions for the deficit as in Table 8.

Table 8. *Monthly values of deficit ($R - D_{20}$) (from Koshal)*

Jan.	$0.398 + (0.033 \pm 0.038) R' - (0.0023 \pm 0.0129) T'$
Feb.	$0.463 - (0.040 \pm 0.037) R' + (0.0336 - 0.0136) T'$
Mar.	$0.893 + (0.072 \pm 0.056) R' + (0.0538 \pm 0.0242) T'$
April	$1.365 + (0.323 \pm 0.050) R' + (0.0134 \pm 0.0265) T'$
May	$1.578 + (0.389 \pm 0.041) R' - (0.0633 \pm 0.0228) T'$
June	$1.627 + (0.455 \pm 0.058) R' + (0.0204 \pm 0.0419) T'$
July	$1.965 + (0.504 \pm 0.040) R' - (0.0071 \pm 0.0244) T'$
Aug.	$1.936 + (0.306 \pm 0.049) R' - (0.0200 \pm 0.0341) T'$
Sept.	$1.485 + (0.271 \pm 0.037) R' - (0.0583 \pm 0.0263) T'$
Oct.	$1.331 + (0.123 \pm 0.031) R' + (0.0006 \pm 0.0198) T'$
Nov.	$0.652 + (0.029 \pm 0.028) R' - (0.0147 \pm 0.0137) T'$
Dec.	$0.402 + (0.015 \pm 0.027) R' - (0.0170 \pm 0.0140) T'$

The non-significant regression coefficients are given in italics; those which are significant are in ordinary type, and it will be seen that the temperature terms are generally non-significant and not all of the same sign. The rainfall terms fall into two groups. Five winter months have non-significant coefficients, and if to these we add October as having the smallest coefficient of the remainder, the year can be divided into two equal periods for which we may equate the deficit and evaporation with some confidence. Let us compare the parts with the whole.

Annual evaporation

If all the monthly expressions are taken together to give a value of the deficit for the whole year we obtain, neglecting standard errors, an equation containing only R' :

$$E_{an.} = R - D = 14.10 + 0.20R',$$

where R' is now the deviation from the mean *annual* rainfall. This equation summarizes Miller's observations, the difference between annual rainfall and annual drainage being very nearly constant, but tending to increase with annual rainfall. Fig. 6 shows the data for the period covered by Koshal's analysis. There is a considerable scatter of the points, but the positive regression coefficient is apparent. The data for the two halves of the period have been kept distinct and there is evidence of a secular change in the deficit. The values for the earlier

period tend to be greater than those for the later period, indicating that the deficit has tended to decrease with time, or, from the view point of Russell and Koshal, annual drainage has tended to increase with time.

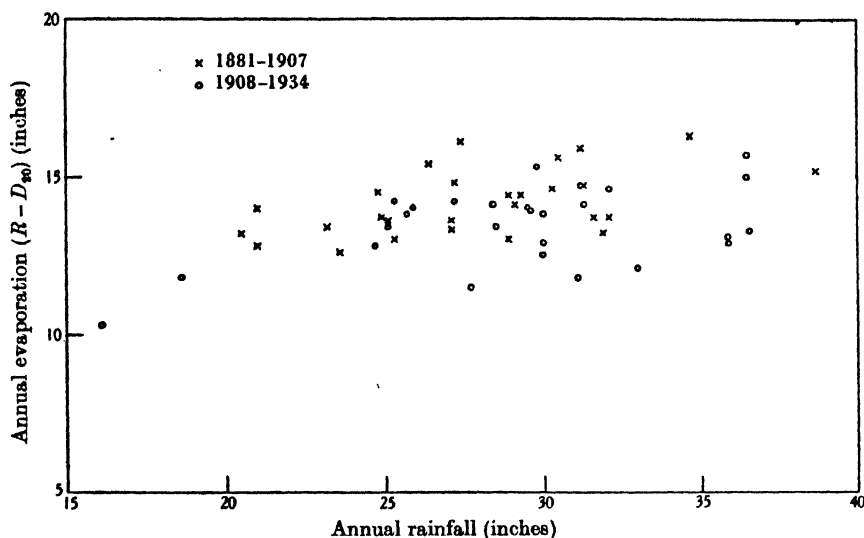


Fig. 6. Annual evaporation from fallow soil (Rothamsted).

Summer and winter evaporation

The data for the 6-month periods are shown in Fig. 7. The points for the two parts of the 50-year period are again distinguished and the figure gives a general picture of the difference in the influence on evaporation of summer and winter weather. A line of unit slope has been included ($E = R - D = R$) which represents arid conditions in which there is no drainage. No points can be above this line. The figure shows several interesting points:

- (1) As one would expect, evaporation is greater in summer than in winter.
- (2) There is a positive regression on rainfall for the summer months and a non-linear regression equation would be needed to fit the data.
- (3) The winter points show the anticipated independence of rainfall.
- (4) The summer scatter is much less than that for winter and it is probable that most of the summer scatter is due to distribution of rainfall. Rainfall distribution in winter is of little importance and other reasons for the winter scatter must be sought (p. 92 above).

(5) There is no evidence of secular change in either winter or summer data; the points for the two 25-year periods are well mixed, parallel

to the $R-D$ axis; there is, however, bias toward heavier winter falls and lighter summer falls in recent years.

In an analysis of the Rothamsted rainfall data for 1854-1929, Wishart (1930) found that the winter rainfall (November-April) had increased and that of other months had diminished. As Fig. 7 shows, most of the winter rain appears as drainage, and if an increasing fraction of the annual rainfall occurs in winter, the annual drainage will increase. Thus the secular change in annual evaporation may be entirely due to the secular change in rainfall distribution. It provides no clear evidence of a physical change in the gauge, though such a change may have occurred.

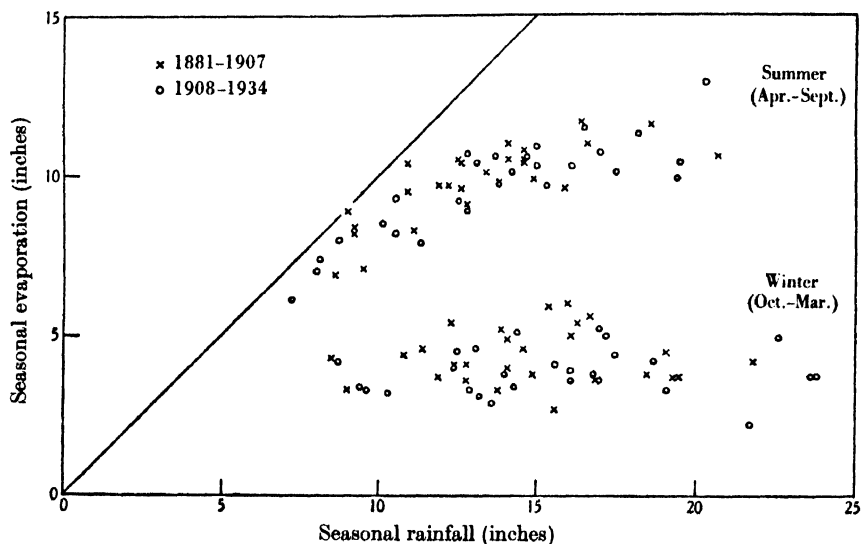


Fig. 7. Summer and winter evaporation from fallow soil (Rothamsted).

The evidence of the automatic records suggested that there is no marked change in field capacity between winter and summer. If there were such a change it might account for part or all of the scatter in the winter data, as one would expect marked differences between the deficits for winters following dry summers and for those which follow wet summers. Table 9 shows the deficits for winters following summers with (a) $\sum R_s < 11$ in., and (b) $\sum R_s > 16$ in.

The mean summer rainfall for the second group is almost twice that of the first, and remembering that the natural periods to which these are approximations will tend to include more dry days at the end of September in the first than in the second group, one would expect the

“dry summer” deficits to be slightly greater than the others. Even without this qualification, however, the difference is not statistically significant, although it is of the expected sign.

Table 9. *Winter deficits after dry and wet summers*

(a) Dry, $R_s < 11$ in.			(b) Wet, $R_s > 16$ in.		
Year	R_s	$(R - D)_w$	Year	R_s	$(R - D)_w$
1887	9.194	3.720	1880	17.065	3.699
1893	8.996	5.567	1882	16.368	4.174
1898	9.197	4.612	1888	16.550	4.086
1900	10.945	5.192	1889	18.642	3.560
1901	9.480	4.407	1903	20.667	5.567
1904	10.885	4.554	1912	17.524	4.119
1906	8.558	5.449	1917	19.443	4.472
1913	10.532	3.925	1918	18.216	3.323
1914	8.741	3.694	1920	16.122	3.327
1921	7.232	4.638	1922	16.554	3.113
1928	10.069	4.003	1924	20.285	4.190
1929	7.997	4.923	1927	19.517	3.783
Means	9.33	4.557 ± 0.608		18.08	3.951 ± 0.630

Difference in mean deficit = 0.606 ± 0.875 in.

We conclude (i) that the seasonal totals show no significant effect ascribable to a change in gauge structure, and (ii) that the cause of the winter scatter must be sought elsewhere.

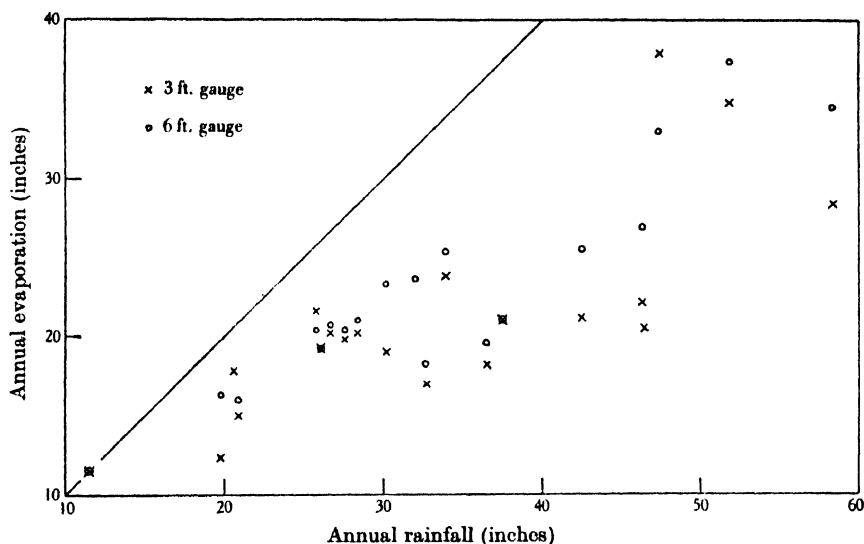


Fig. 8. Annual evaporation from fallow soil (Cawnpore).

For comparison with the Rothamsted summer data we show in Fig. 8 the annual data for Cawnpore (Leather, 1911; Batham, 1925) which should show similar features. The points are more scattered, as the rain-

fall is much more erratic in amount and distribution, but the positive regression of deficit on rainfall is apparent. On general grounds one can see why the regression line should be curved. Assuming that the rainfall is spread uniformly over the period, in very dry seasons it will all be evaporated and the points will lie on the line of unit slope. As the rainfall increases or the regularity of precipitation decreases, there will be periods in which all the rain of one fall has not been evaporated when the next fall occurs and a little will appear as drainage. As the number and intensity of the falls increases, more will be evaporated and more will come through as drainage, i.e. the curve moves away from the line of unit slope. In the limit, if there were continuous rain throughout the period, all the rain would appear as drainage, i.e. the curve would turn back and reach the *R*-axis.

Monthly values of deficit

Up to the present we have discussed evaporation during long natural periods. As shown above (p. 88) we cannot consider calendar months as such but we can discuss the modified form of Koshal's equations (p. 96) and re-examine his conclusions. The main conclusion, apart from those on secular changes and gauge differences which have already been considered, is, that because mean air temperature does not appear as a significant variable, the difference between winter and summer drainage, usually ascribed wholly to difference in evaporation, must be in part accounted for by accumulation of water in the gauges. The latter we reject because the evidence of the automatic charts and that of the effect of summer rainfall on winter deficit agree in showing that there is no significant seasonal change in gauge structure. There are two other possibilities which we shall discuss in order. The first is that the analysis was such as to conceal the effect of temperature, and the second is that mean air temperature is useless as a measure of evaporating power.

Koshal's periods were calendar months and not natural periods, and we have already outlined the nature of the errors to which this discrepancy might lead. We have also seen that in summer months, when large temperature effects are to be anticipated, that rainfall distribution has a pronounced effect on drainage, and Fig. 7 showed that the regression on rainfall in summer was not linear. These objections may not be serious, but they do raise doubts about the treatment of the temperature effect.

In any case Koshal's equations do show the effect of the increased evaporation in summer. Each month has a constant term which varies cyclically throughout the year, and these terms are the 50-year mean

values, i.e. R' and T' both equal to zero. As long period means we may consider them as arising from natural periods and see upon what factors they depend. This is the starting point of Crowther's analysis. His equation was obtained from the mean of all three gauge records so that the data were slightly different, but in effect, it was derived from the constant terms in Koshal's equations. His equation can be rearranged to read

$$R - D = 1.147 - (0.112 \pm 0.133) R_1 + (0.069 \pm 0.006) T_1,$$

where R_1 is the deviation from the mean monthly rainfall (2.44 in.), and T_1 is the deviation from the mean monthly temperature (48.1° F.). In this equation the term in R_1 is not significant, and rounding off the remainder, we have

$$E = R - D = 1.15 + 0.07 T_1$$

for any calendar month.

It is obvious that neither Koshal's nor Crowther's treatment is quite adequate by itself, always assuming that temperature is an important variable. Following this review is a note by Mr Sahni in which natural periods and a non-linear regression on rainfall are used. The results confirm those obtained by Koshal in showing no significant dependence of evaporation on mean air temperature but there is some evidence that low relative humidity and high wind velocity may facilitate evaporation.

We must, therefore, fall back upon the alternative possibility and examine in some detail how far the assumption is justified that mean air temperature is the most important single variable determining evaporating power.

FACTORS AFFECTING EVAPORATION FROM SOIL

The most striking difference between the analyses of Crowther & Koshal is that the former found a significant correlation of evaporation with mean monthly air temperature, whilst the latter's results for individual months showed little dependence on this factor. Crowther's equation expresses the common knowledge that evaporation varies cyclically throughout the year, and a significant correlation would be obtained with any other cyclic variable; Koshal's equations suggest that mean air temperature is not the best variable to choose. This can be seen if we plot mean monthly $R - D$ against T ; a fairly regular change takes place from month to month, the series of twelve points forming a loop in which the autumn values of $R - D$ are smaller than those for spring at the same mean temperature. Thus at 42° F., the monthly deficits are: 1.25 in. in spring; 0.85 in. in autumn.

Evaporation is due to a mass transfer of water vapour under a partial pressure gradient, and from soils, may be considered in three stages.

(a) *Movement in the soil.* If evaporation is to proceed steadily at the soil surface the water supply there must be replenished either by rain or by upward movement from below. The effect of rain has already been discussed and we consider the other means of supply. If, due to viscous resistance in the soil, the upward liquid movement is not sufficiently rapid, the rate of surface evaporation will decrease as the layer at 100 % R.H. retreats below the surface. Evaporation will then depend upon the resistance to the diffusion of the vapour through the air-filled pore space of the soil, and on the depth of the 100 % R.H. layer, i.e. upon the viscous resistance to liquid movement in the water-filled pore space. Prolonged drying thus sets up two kinds of resistance to evaporation, and only when the surface is kept continuously moist will evaporation be similar to that from an open water surface.

(b) *Diffusion through a still layer of air, and (c) turbulent mixing with the atmosphere.* In general there will be a layer of still air immediately above the soil (or water surface), of variable thickness, l , across which there is a vapour pressure drop, $p_s - p_a$, and the water vapour flow per unit area will be given by

$$dq/dt = D_0(T/273)^2 (p_s - p_a)/l,$$

where D_0 is the coefficient of diffusion of water vapour into air at 0° C. The value of l will depend upon wind velocity and steadiness, and the constancy of p_a will depend upon adequate mixing of the diffused vapour with the air above. It is probable that there is usually sufficient breeze to ensure that this turbulent mixing is complete, and stage (c) will not be further considered. p_a may therefore be taken as given by the air conditions several feet above the soil surface. The value of l will tend to decrease with increased wind velocity, but as it is of the order of $\frac{1}{4}$ cm. the stone coping round the gauges (10 cm. high) will act as a wind screen and we cannot predict how changes in wind velocity and direction will modify it. For the remainder of the discussion we assume that l remains constant and we have as a measure of the rate of evaporation the function, $(T/273)^2 (p_s - p_a)$, in which the temperature term is not very important. The annual cycle of evaporation is, therefore, primarily dependent upon changes in $p_s - p_a$. The value of p_a is the vapour pressure above the still layer and is given by (i) R.H. of air and saturation vapour pressure at the air temperature, or (ii) saturation vapour pressure at the dew point. The value of p_s is the water vapour pressure at the soil

surface, and when the surface is at 100 % R.H., p_s is the saturated vapour pressure of air at the *temperature of the soil surface*. To obtain an estimate of p_s we make two assumptions about soil surface conditions. (a) The moisture content is assumed to be sufficient to keep the air in the surface at 100 % R.H.; (b) the mean surface temperature is given by the mean air temperature (T). We shall discuss later the effects of the errors introduced by these approximations. They lead to an expression for the evaporating power in terms of air conditions:

$$\text{Air evaporating power} = (p_T - p_a) (T/273)^2 = p_T(1.00 - \text{R.H.}) (T/273)^2,$$

where p_T is the saturated vapour pressure of air at T° K. Although $p_T \neq p_s$, it seemed worth enquiring how evaporation varies with

$$(p_T - p_a) (T/273)^2.$$

In Fig. 9 have been plotted the daily rates of evaporation against the evaporating power. The ordinates have been obtained from the constants of Table 8 and represent $R - D_{20}$ divided by the number of days in the month: these are means for the period 1878–1932. p_T and T have been obtained from the corresponding data (Koshal's Table 1), but the R.H. is the monthly mean for the 11-year period 1927–37. The variance in R.H. for a given month is not very great and the 11-year means are probably a good index to the corresponding means for the longer period. Koshal's equations have been used to correct the soil ordinates to the standard rainfall of 2.431 in. per month. The correction chiefly affects the summer months, for three of which a Sahni correction may also be made by extrapolating his data for shorter periods. To indicate the order of magnitude, the measured value, the Koshal corrected value and the Sahni corrected value are given (in. per day) for June and July, months with mean rainfall respectively less and greater than the annual monthly mean:

	Measured $R - D$	Koshal corrected	Sahni corrected
June	0.0542	0.0583	0.0602
July	0.0634	0.0594	0.0586

The Sahni correction is the greater, and as it may not be safe to extrapolate his data to 30-day periods, the Koshal-corrected points have been used in drawing the curve. This is smooth and shows no trace of a loop, i.e. the rate of evaporation is a unique function of evaporating power which is thus a more satisfactory parameter than mean air temperature. On the figure have been plotted data for the rate of evaporation from an open water surface. They have been derived from

tables given by Latham (1909) and are 30-year means (1879-1908). The evaporating power is calculated from the temperature of the water in the evaporation tank and that of the dew point. There are several approximations involved, similar in kind to those made in computing the soil data. The readings are 9 a.m. values and not true daily means;

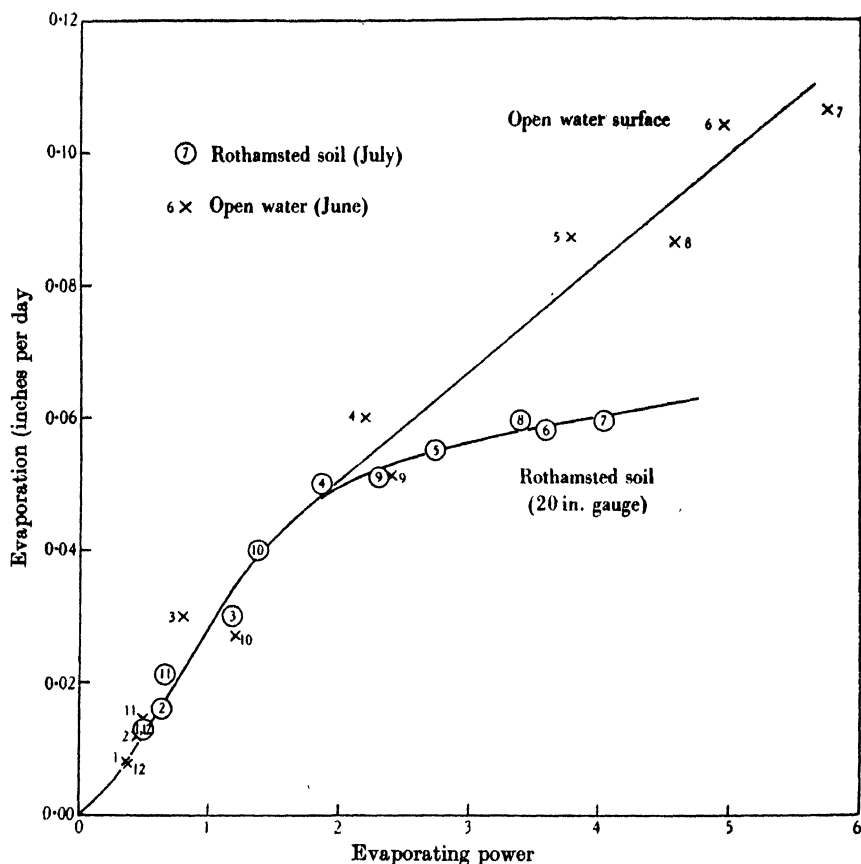


Fig. 9. Seasonal variation of mean daily evaporation from open water and fallow soil.

the effects of sunshine are ignored and will be most marked in summer when the sun warms the bottom of the tank; the water temperatures are not surface temperatures, and anomalous effects might be anticipated in winter when the air temperature is below 4° C. Finally, there may be a rainfall correction necessary, as the changes in tank level were compensated for increments due to rain, presumably measured on a rain gauge of smaller area. The Rothamsted 1/1000 acre rain gauge usually

records up to 10 % more rain than the 5 and 8 in. gauges and it may be that Latham under-estimated the amounts added in rain.

The winter groups of the two sets of data are fitted by the same curve, which, except near the origin, is a straight line, indicating that during the six months October to March the evaporation from fallow soil is practically the same as that which would take place from an open water surface exposed to the same meteorological conditions. There is no extensive drying of the surface during this season and between falls of rain the water supply from below is sufficient to keep the surface at 100 % R.H.

If the abscissa were an exact measure of $(p_s - p_a) (T/273)^2$ one would expect the open water curve to be a straight line passing through the origin. The curvature near the origin is probably due to the surface temperatures being less than that of the air; the surfaces will tend to take up the wet bulb temperature, and thus the winter evaporating power will be overestimated. In the water tank, convection will normally tend to equalize temperatures, but this action will be ineffective during long periods in winter when the air temperature is below 4° C. In the soil, the variations in soil surface temperature are probably similar to those observed by Keen & Russell (1921) at 6 in. They found that the winter soil temperatures at these depths were generally lower than, and out of phase with, the air temperature. Although the soil surface and the air form parts of a coupled system whose temperatures must be inter-dependent, it is apparent that in winter months a small change in either temperature may produce a relatively large change in evaporating power, and because of phase differences in the diurnal variations very different amounts of evaporation will be possible in periods which have the same rainfall, mean air temperature, mean relative humidity, and mean wind velocity. Hence the large scatter in the winter points in Fig. 7.

During summer months the evaporating power is sufficiently large to cause drying out of the soil surface between showers. The evaporating power will be greater than has been calculated because the mean soil surface temperature is higher than the mean air temperature by an amount which is probably dependent on the amount of sunshine. The records of Keen & Russell show that this temperature difference is marked at 6 in. While the surface is wet after a shower, evaporation will be rapid and the layer of 100 % R.H. will quickly retreat into the soil, so that for the greater part of summer months the resistance to evaporation imposed by the soil may be as great or greater than that due to the still layer. Fluctuations in air temperature will then have very little

effect and the total amount of evaporation in a summer period will be primarily dependent upon the number of times the surface is wetted during the period; in general this means that the more rain there is, the greater will be the total evaporation, but, in months of equal rainfall, there may be wide variations depending upon rainfall distribution. Corrections for variations in meteorological conditions will, therefore, have little or no effect on the scatter of summer values of evaporation (Fig. 7). Thus in Sahni's analysis of the residuals the minor role played by atmospheric conditions is reflected in the insignificant correlation with mean air temperature and the indeterminate nature of the regressions on humidity and wind velocity.

The conditions necessary for a successful description of evaporation from soil are now clear. Although other variables are important, the primary objective must be the evaluation of the term $(p_s - p_a)$ in the general diffusion equation (p. 102); the winter data of Fig. 9 show that where a reasonable rough approximation can be made the dependence of the rate of evaporation on evaporating power is as expected. Even in summer, where the approximation ceases to be reasonable, the evaporating power is still a more useful parameter than mean air temperature if empirical correlations are being sought. The inadequacy of the summer approximation may be regarded as due either to the retreat of the layer at 100 % R.H. into the soil, so introducing a soil resistance to evaporation, or to the reduction of the R.H. of the soil surface air below 100 %; from both conceptions one finds that p_s is less than its maximum value—assumed maintained in calculating evaporating power—for long periods in summer. The direct measurement of p_s and p_a will be difficult; an indirect approach through measurement of temperature and humidity of the air in and above the soil surface may be possible. This will involve four variables instead of the two used by Crowther and Koshal and until all four can be included in a statistical survey incorrect conclusions may be drawn from analyses based on partial knowledge of the factors involved.

SUMMARY

1. Study of the automatic records shows:

(a) There is a seasonal change in the drainage response after rain which can be almost wholly ascribed to viscosity changes arising from seasonal changes of soil temperature (p. 77).

(b) Evaporation occurring after a fall of rain has no measurable effect on the drainage response to that rain (pp. 78, 87).

(c) The maximum drainage rates for the 20 in. gauge are much larger than those for the deeper gauges. The maxima change seasonally and are again primarily dependent on viscosity (p. 78).

(d) There is no marked change in the field capacity of the gauge during the year. The air-filled pore space at field capacity may change by about 15 % of its average value (p. 81).

(e) The air-filled pore space at field capacity averages about 1.6 % (0–20 in.) and 0.25 % (20–60 in.). This order of magnitude is confirmed by measurements of Lawes & Gilbert (p. 81).

(f) Drainage begins some time after rain starts, the delay being normally due to (i) the need to bring the soil up to field capacity, and (ii) the finite time required for water to move through the gauge (p. 83).

(g) Abnormal drainage may occur when (i) rain falls very heavily and air is entrapped, or (ii) the soil is partially frozen (pp. 84, 86).

(h) Natural periods can be chosen over which $R-D$ can be equated to the evaporation. Good approximations are: periods of a year, six months, or long series mean of individual calendar months. A single calendar month will rarely approximate to a natural period (p. 87).

2. The daily totals reveal:

(a) The 20 in. gauge rarely loses more than 0.7 in. of water by evaporation. This corresponds to an air content of 5 %. The corresponding figure for cultivated soil is *c.* 8 % (p. 90).

(b) Most of the summer evaporation occurs soon after the end of the rainfall (p. 91).

(c) Total summer drainage is very much dependent on rainfall distribution; winter drainage is little affected (p. 92).

3. There is a leak in the casing of the 40 in. gauge. There may be a slight fault in the walls of the 60 in. gauge (p. 93).

4. A brief survey of the Pusa daily totals indicates that drainage is facilitated by root-holes, etc., the rate of drainage appears to be slower than at Rothamsted, and the extent of the water loss between monsoons very much greater (p. 94).

5. The seasonal totals show:

(a) Annual evaporation is nearly constant; it increases with increasing rainfall (p. 96).

(b) Summer evaporation is about two to three times as great as that in winter; it is dependent upon rainfall whereas winter evaporation is independent of rainfall.

(c) There is no evidence of a secular change in the seasonal evapora-

tion; a secular change in the annual evaporation is due to a change in rainfall distribution (p. 98).

(d) Winter evaporation is not dependent upon the nature of the preceding summer weather (p. 98).

(e) The equations of Koshal and Crowther are reconsidered as regressions of $R - D$ (deficit) on R and T . They are shown to be complementary and indicate a seasonal change of deficit. The use of natural periods does not improve the significance of the Koshal regression on mean air temperature. His explanation of the non-significance is rejected (p. 100).

6. Evaporation is discussed on a physical basis in terms of the water vapour pressure gradient in the air immediately above the soil surface:

(a) In winter the soil does not dry at the surface. Winter evaporation is, therefore, much the same as would be obtained from a water surface and extra rainfall does not affect it.

(b) In summer the surface remains moist only for a short time after rain has fallen; the air gradient is then much steeper than in winter. For the rest of the time the surface is drier and there is also a vapour pressure gradient in the soil. Hence (i) there is more rapid evaporation while the surface is wet, (ii) the total amount of evaporation is dependent upon both total rainfall and on its distribution in time, (iii) the later stages of evaporation are more dependent upon soil conditions than on air conditions, and (iv) the total evaporation is much less than from open water (p. 105).

(c) An adequate description of evaporation in all seasons may be obtained from knowledge of $(p_s - p_a)$, the difference in the partial pressures of the water vapour of the air in and above the soil surface. This term is the most important in the general evaporation equation

$$dq/dt = D_0(T/273)^2 (p_s - p_a)/l,$$

and the apparent anomalies in previous statistical treatments are attributed to the impossibility of representing changes in $(p_s - p_a)$ in terms of changes of rainfall and mean air temperature alone.

Our survey, although covering broadly the period 1870-1940, has been chiefly concerned with the records of more recent years, during which the observations have been in the care of Mr W. C. Game. To him and to his various assistants during the past 30 years we offer our thanks for the consistently high standard of observation they have maintained.

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LABORATORY EXPERIMENTS ON EVAPORATION FROM FALLOW SOIL

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(With Three Text-figures)

PREVIOUS field studies (Penman, 1940*a*; Penman & Schofield, 1941) have held out the promise that evaporation rates might be physically interpreted in terms of normal meteorological data supplemented by certain soil data, of which the chief is the value of the water vapour pressure at the soil surface. While the surface is moist it is sufficient to know the surface temperature; when the surface is dry there is no simple way of obtaining the required information. Pending the development of a suitable method there is much semi-empirical information about evaporation processes that remains to be found and the experiments described below are an attempt to fill in some of the gaps in our knowledge. There are three main groups: (1) Evaporation under isothermal conditions, in which, apart from surface cooling produced by evaporation, the soil is kept at air temperature. (2) Evaporation under simulated summer field conditions in which the soil surface is maintained at a higher temperature than the air for part of the day. (3) The effect of dissolved salt in the soil water is studied under both isothermal and non-isothermal conditions.

GENERAL EXPERIMENTAL DETAILS

The experiments were carried out in a constant temperature room, ventilated from outside the laboratory and thus of variable humidity. A constant-speed ceiling fan provided a steady breeze estimated at about 9 miles/hr. over the small area employed in the experiments. Two types of soil have been used in 12 in. cylinders freely drained. Rothamsted allotment soil—a clay loam—was taken in its field condition in Spring, being transferred to the cylinders a little at a time with tamping down after every fresh addition. Woburn soil—a sandy soil—was packed in an air dry condition, with similar small additions and thorough tamping after each. As the cylinders varied in diameter from 5.2 cm. (Woburn soil) to 10.0 and 11.2 cm. (Rothamsted soil) a correction for differences

was made from $E_1/E_0 = (d_1/d_0)^{1.56}$ (Powell, 1940), the standard diameter being that of an open-water surface ($d_0 = 9.6$ cm.). The measured evaporation from open water integrates the effects of air factors (wind speed, relative humidity and temperature), and thus permits a closer study of the effects of soil factors and of radiation on the evaporation process. The open water evaporation also provides a time scale. When needed, surface heating was produced by a 750 W. electric radiator suspended about 2 ft. above the soil surface; the open-water conditions were isothermal throughout.

The general scheme of the experiments is similar to those of Buckingham (1907) but with important changes in detail. There is here no water table maintained at 4 ft., the heating of the surface by radiation is an improvement on his heating band round the top 2 in. of metal cylinders, and the open water surface control is used here in a way which makes the study much more quantitative than Buckingham found possible. In group (2) experiments the radiator was on for about 8 hr. a day and its effect was to raise the surface temperature of dry soil about 10° C. above air temperature. This figure was chosen after making field measurements of soil surface and air temperatures during a week of cloudless anti-cyclonic weather in June 1940. Soil evaporation losses were obtained by weighing the cylinders once or twice daily, to the nearest $\frac{1}{2}$ g. (in 5000 for Rothamsted soil, in 3000 for Woburn soil). Open-water losses were similarly measured to the nearest $\frac{1}{2}$ c.c., the normal daily evaporation being about 40 c.c. although extremes ranged from 1 to 150 c.c./day. For presentation all readings have been converted to inches of water. Thermometers with their bulbs either just below the water surface, or pressed into the soil surface, gave a mean value of the temperatures of surface layers; the wet-bulb temperature of the air was also measured.

EXPERIMENTAL RESULTS

Evaporation under isothermal conditions (Fig. 1). A wide range of drying conditions was obtained by varying air temperature, fan-speed and humidity. The lowest rate of 1 c.c./day (not represented graphically) was obtained by covering the tops of the Rothamsted soil cylinder and the open water tray with lids pierced by three holes c. $\frac{3}{16}$ in. diameter. The curves fall into two distinct groups. (1) The upper group is characterized by an extensive range of approximately unit slope. For each of the soils and for a drying power range of up to 0.22 in. per day (mean air temperature $\leq 20^\circ$ C.) the evaporation from the soil is equal to that from

the open water up to about 1 in., at which a slow decrease in the relative rate is apparent. This change in drying rate coincides with the first visual evidence of surface drying, although shrinkage was apparent at an earlier stage in the Rothamsted soil. The results of the slow evaporation experiment on Rothamsted soil are, for various reasons, not so confidently reduced to absolute values, but the relative rates during the 5 months of the experiment show the same steady evaporation rate up to about 0.8 in. lost, a slow decrease in the rate again becoming apparent at about 1.0 in. If one makes the reasonable assumption that the initial slope is near unity, then the experimental points up to 1.2 in. lost lie on or near

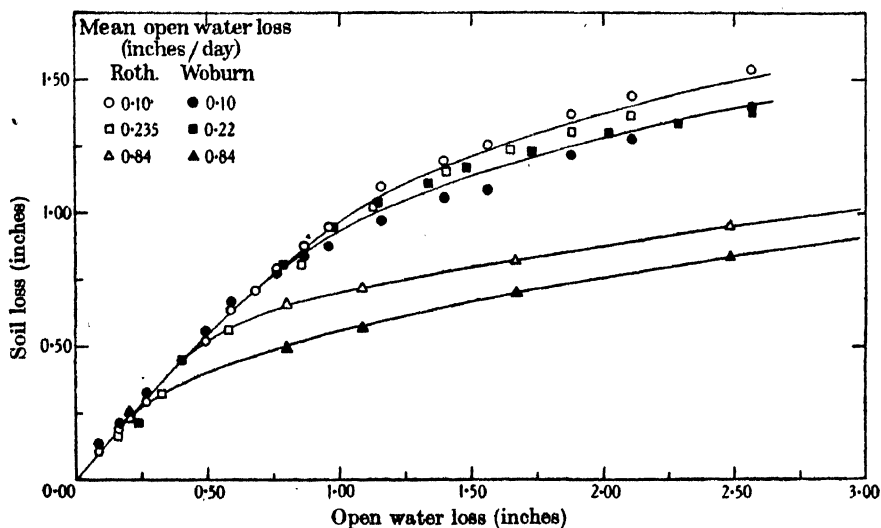


Fig. 1. Evaporation under isothermal conditions.

the mean Rothamsted curve for rates up to 0.22 in./day. (2) In the lower group, obtained with a very great drying power (mean air temperature = 35° C.), the section of unit slope is very short. Surface drying appeared almost complete after 8 hr., the total water loss being then about $\frac{1}{4}$ in.

In both groups the initial drying rate for the sandy soil is greater than for the clay loam, but this order is soon reversed, and eventually the curves run approximately parallel, not only within groups but also between groups.

Conclusions from 'isothermal' experiments. (a) A steady demand on the soil's water supply can be met for a considerable time. The total amount so drawn off is not affected by quite large changes in the rate of extraction.

(b) A very severe demand cannot be steadily met.

(c) The extent of (a) indicates a considerable amount of liquid movement to the surface in spite of the absence of a nearby water-table.

(d) An open water loss of 0.10 in./day corresponds to average English June meteorological conditions. The soil loss in 10 days was 0.94 in., the surface being still dark and moist. This quantity is appreciably more than evaporates from fallow field soil in the same time, and under normal June conditions the soil surface does not remain dark and moist for more than two or three days after rain.

(e) The liquid movement of (c) appears to have a limiting velocity which prevents it from keeping pace with an extremely rapid rate of withdrawal of moisture. Under the conditions of the lower experiments the soils could be described as self-mulched.

Evaporation under simulated summer conditions (Fig. 2). One cylinder of each soil was radiated for 8 hr. a day and kept under steady conditions for the remainder. During the first 2 days weighings were made at the beginning and end of the radiation period but thereafter only one weighing per day was made. Another pair of cylinders was mulched to a depth of c. 1 in. at the beginning of the experiment (before the soil was really in a fit state for this operation). The figure includes the isothermal curves from Fig. 1 for corresponding air conditions.

Before drawing conclusions, certain reservations must be made. Experiments of this type are necessarily qualitative since there are no real standard conditions, and, in the case of the mulching experiments, no reproducibility of conditions. It is thought that the radiation experiment does faithfully simulate certain summer conditions under which possible control of surface evaporation is of technical importance, but the mulching experiments are much less satisfactory. Apart from varying the depth of mulching and the epoch at which it is carried out, they involve a considerable change in the surface geometry, a change that is large in comparison with the size of the surface. The turbulence thereby introduced into the moving air means that the open-water evaporation ceases to be a measure of the drying power of the air passing over the mulched surface.

In a third set of experiments, not represented diagrammatically, mulched cylinders were radiated, the mulching being carried out within an hour or two of the beginning of the first radiation period. For Woburn soil the later portion of the 'mulched and radiated' curve lay above the 'radiated' curve, while for Rothamsted soil it lay slightly below the 'radiated' curve. *For the conditions of the present experiments we can say*

that while there are differences in the early days, after the equivalent of 25 average June days (i.e. 2.50 in. evaporated from open water) the conservation effected by mulching is inappreciable, whether it be impressed on isothermal or radiated conditions.

For the remainder of this section attention is directed to the intermittently radiated soils. No significance can be attached to the differences between the Rothamsted and Woburn curves as removal and replacement of the cylinders for weighing probably involved day to day changes in the relative intensities of radiation falling on the surfaces. The results

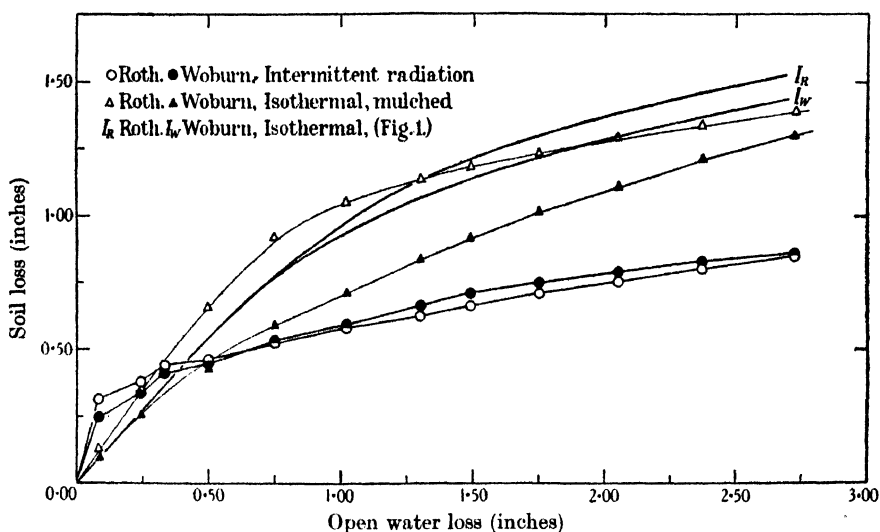


Fig. 2. Evaporation under simulated summer conditions.

show the expected high initial rate of evaporation, a rapid decrease in the rate coinciding with obvious surface drying on the second day, an early intersection with the isothermal curve, and thereafter an almost constant drying rate of the same order as that of the later stages of isothermal drying.

Conclusions from radiation experiments. (a) Rapid initial drying conditions are maintained for only a short period; this period lasts as long as the soil surface is moist, i.e. about 2 days.

(b) Thereafter the rate of loss is nearly constant and there is clear-cut evidence of conservation as compared with isothermal evaporation.

(c) The total loss after the equivalent of 25 June days is c. 0.85 in., of the same order as is found in field experiments. This, with (a), indicates that the intermittent radiation has effectively reproduced summer field behaviour.

(d) Roughly: the total evaporation after t days under radiative conditions is given by $E \approx at^{1/n}$, where $n \approx 3$.

Effect of dissolved salt (Woburn soil) (Fig. 3). Of four cylinders, two were leached with $N/10$ NaCl, the others as usual with tap water. The cylinders, A , B , C and D were treated as follows:

A , Isothermal: salt	C , Radiated: salt
B , Isothermal: no salt	D , Radiated: no salt.

Midway through the first radiation period θ_A —the surface temperature—was greater than θ_B (20.4 , 20.0° C.), and θ_C greater than θ_D (27 , 26),

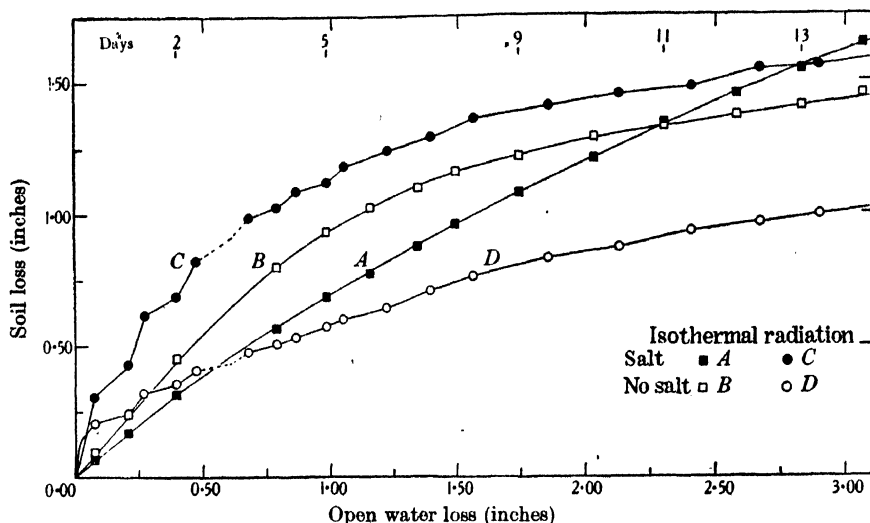


Fig. 3. Effect of dissolved salt on evaporation (Woburn soil).

indicating more rapid evaporation from the surfaces of B and D . At the end of the first radiation period, θ_D was greater than θ_C (30.5 , 29), indicating a change-over in the relative evaporation rates from these radiated surfaces. This evidence justifies the intersection in the curves of C and D between the origin and the first experimental point. Attention is directed to several points of interest.

Initial slopes. As before, we have the general difference between radiated and isothermal conditions, but in each group we see the effect of decrease in vapour pressure due to the dissolved salt.

Subsequent behaviour and intersections. Although the initial 'salt' slopes are less than the others, the reduced rate of evaporation is maintained for a longer period. Thus, while B and D intersect on the second day (cf. Fig. 2), A and C only intersect after 13 days. C and D do not

intersect again nor does *C* intersect *B*; actually in the later stages all three are approximately parallel, showing that under what we may now call normal summer field conditions the evaporation from a saline soil is much greater than from a non-saline soil, and is also greater than from a non-saline soil under isothermal conditions.

Both *A* and *C* showed salt efflorescence after about 10 days, indicating conditions of saturation at the surface. *A* was still dark after 15 days of drying.

Conclusions from salt experiments. (a) The presence of salt in the soil water depresses the initial rate of evaporation, but this decreased rate is maintained for a longer time. Under isothermal conditions one would expect curve *A* to be similar to curve *B* if the open water could have a salt content continuously adjusted to the same concentration as the soil moisture at the surface of cylinder *A*.

(b) Under radiative conditions the supply of liquid to the surface is maintained for a length of time comparable with that for isothermal non-saline conditions. This liquid flow will be of a salt solution and hence a salty patch of soil will (i) continue to evaporate water and remain more moist than neighbouring less salty patches, and (ii) will tend to become saltier at the expense of those neighbouring patches.

Miscellaneous experiments. (1) In each of the figures attention has been drawn to the approximate equality of slope in the later stages. An experiment was performed with Rothamsted soil in which intermittent radiation was maintained until the isothermal curve was intersected; thereafter isothermal conditions were maintained. Compared with an experiment in which intermittent radiation was maintained throughout, the curves ran very nearly coincident and parallel from this point of intersection onward. Thus for a 3-day period the following figures were obtained.

	Mean air temp. ° C.	Mean wet- bulb temp. ° C.	Soil loss g.	Water loss c.c.
Isothermal after intermittent radiation	20.9	17.3	31	122
Intermittent radiation throughout	21.2	17.8	32	130

Ratio of slopes = 1.03.

The cumulative evidence suggests that some equilibrium is eventually attained whatever the nature of the initial behaviour, and that the differences between isothermal conditions, intermittent radiation, and mulching lie in the rapidity with which this equilibrium rate is attained and the gross amount of water lost in attaining it.

(2) The Woburn cylinders used in the salt experiment, and a contemporary Rothamsted isothermal *v.* radiated test were covered up at the end of 15 days drying and left for a fortnight; apart from a slight leakage through imperfect seals there was no further evaporation. The following details were recorded before and after this rest period:

		Appearance	
		2. viii. 40	14. viii. 40
Woburn	Cylinder <i>A</i>	Dark: efflorescence of needle-like crystals	Dark and moist: no efflorescence
	<i>B</i>	Light	Dark but not obviously moist
	<i>C</i>	Light: (efflorescence blown away)	Darker than <i>B</i> but lighter than <i>A</i>
	<i>D</i>	Light	Approx. same as <i>B</i>
Rothamsted	Glass	Light: cracked: dry down to $\frac{3}{4}$ in. next wall	Dark and some parts moist: no dry layer
	Copper	Light: no cracking but shrunk from wall	Dark: more uniform than glass cylinder

There is evidence of redistribution of moisture here. All surfaces showed a darker appearance, the salt efflorescence disappeared, presumably by re-solution and diffusion away from the surface, and in the only transparent-walled cylinder the dry surface layer was no longer to be seen. The drying was then resumed under isothermal conditions. After 1 day, *B*, *C* and *D* had settled down to a steady drying rate, and in 2 days the others reached a steady rate, the surfaces being light and dry, and in the glass cylinder the dry $\frac{3}{4}$ in. layer was again apparent. An estimate of the amount of water redistributed in the rest period was obtained by subtracting from the total evaporation of the first 2 days the amount evaporating in 2 days at the ultimate steady rate. For the glass cylinder this amount was $28 - (2 \times 5\frac{1}{4}) = 16\frac{1}{2}$ g., i.e. 0.085 in. of water; the evaporation rate before the rest period was 0.045 in./day. Assuming that at the beginning of the rest period the 100% R.H. layer was 2 cm. deep, a rough estimate based on a diffusion equation previously developed (Penman, 1940*b*) indicates that diffusion of vapour would account for about 95% of this redistribution during the rest period, i.e. it may not be necessary to assume that any appreciable movement as *liquid* had taken place in spite of the pronounced moisture gradient in the soil.

GENERAL DISCUSSION

Where comparison is possible the results of the preceding experiments are in substantial agreement with those of Buckingham, the absence of a nearby water-table apparently having no effect on the nature of the

phenomena observed. Buckingham suggested that soils would be 'self-mulching' under arid desert conditions, but the present work suggests that this is still true under normal English summer conditions.

The previously observed features of winter and summer evaporation (Penman & Schofield, 1941) are again found in the isothermal and intermittent radiation experiments respectively; that is, for the former, the soil and open water losses are equal for periods of the order of the normal interval between rainfalls, and for the latter, the soil losses are higher in the early stages and less in the later stages than the open water loss. The result of an incomplete survey of the annual cycle of soil surface and air temperatures indicates that conditions may be regarded as isothermal when the mean air temperature is below 48° F., and as non-isothermal above 48° F. For Rothamsted this means that approximate isothermal conditions exist from October to April inclusive, and during the remaining 5 months we must regard field conditions as similar to those of intermittent radiation. Thus the broad difference between winter and summer evaporation is that between isothermal and radiated conditions.

In general, the condition under which mulching has a beneficial effect on water conservation is that specified by Shaw (1929), namely, that the water-table should be within a few feet of the surface. The preceding experiments suggest an alternative criterion, namely that mulching may or may not be effective according to the time of year at which it is done. Thus King (1890-1) found evidence of conservation by a spring mulch, but his results for summer mulching show no significant effect. Veihmeyer's (1927) Californian summer experiments showed no benefit attributable to mulching. He found that the evaporation loss for 80 days was already half complete in 5 days, indicating a relation of the form $E \propto at^{\frac{1}{2}}$, i.e. of the type already suggested for the radiation curves. It is apparent then that during a long rainless period in summer, i.e. at such a time when possible control of evaporation is important, the effect of the sunshine itself is to produce a surface mulch and cultivation does not appreciably affect its efficiency.

There is abundant evidence from the curves that, except under extraordinary circumstances, a steady drain on the soil's water supply can be met, even in the absence of a nearby water-table. The fact that the grass on the Chalk Downs can remain green during a period of drought, although the water-table is some 200 ft. below (Hall, 1904), is a reflection of the ability of the chalk to retain rain water and of the conservative demands made upon the water by the grass. It is no proof that water was moving upward from a water-table 200 ft. below, and those later

writers who have quoted Hall's tentative suggestion as experimental evidence supporting this idea, have done so in spite of the omission of the relevant paragraph from later editions of his book.

Veihmeyer's experiments, and later work, theoretical and experimental, by Schofield (1935) indicate that flow of liquid water from a moist to a contiguous dry soil depends upon the energy gradient, which may be zero even for a positive moisture gradient. The present experiments are, therefore, to be interpreted by assuming that the action of radiation, or of very high air temperature, is to dry out a shallow layer at the surface more quickly than it can be replenished by liquid flow from below. Once this dry layer is produced water movement from below is entirely in the vapour phase, leading to the following consequences:

(a) Evaporation rates are very much reduced. They are only slightly dependent upon air and soil surface conditions; hence the later slopes of all curves are about the same and correspond to a dry layer of about 3-5 mm. thick. The rate is then about $\frac{1}{20}$ in. per day.

(b) When the evaporation process is suspended, the rate of redistribution in the region of steep moisture gradients is almost entirely dependent upon the rate of diffusion of water vapour.

If there is some other factor tending to restrict the effects of the rapid drying power, then evaporation losses may be very great as in the experiments with salt, where the effect of the reduced vapour pressure was to prevent the formation of a dry layer, so that self-mulching action was not apparent at as early a stage as in the non-saline experiments.

The mechanics of the formation of this dry layer is doubtful owing to our limited knowledge of the dynamics of water movement in soils. In the case of surface radiated soil, downward distillation of moisture will undoubtedly help to dry out the surface layer, but this does not seem to be a necessary condition, since in the high air temperature experiment the soil surface was very much cooler than the bulk of the soil, i.e. distillation would take place into, and not away from, the surface layer. The liquid movement depends upon the capillary conductivity and the suction gradient, both being functions of moisture content; the vapour movement depends upon the relative humidity of the soil air and this is not nearly so dependent upon moisture content as the liquid variables are. Hence it is conceivable that while a large decrease in liquid conductance could take place, the vapour conductance would not change appreciably, so that in the thin surface layer the rate of removal by vapour would exceed, and continue to exceed, the rate of renewal by liquid. The condition produced, in which there is a dry layer with ample reserves of moisture

a few mm. away, seems to depend on the existence of a two-fold mechanism for water movement. In the region round a plant's root the plant might produce a similar state of affairs by transpiring so rapidly that it formed a barrier between the roots and the water supply they needed, causing wilting within a short distance of an irrigation channel.

One other point of physical interest deserves mention here. In discussing the isothermal curves we have taken the open water and soil surface losses as equal. In magnitude the slopes are slightly greater than unity but as the temperature of the soil surface was invariably higher than that of the water surface, for the same vapour pressure difference the soil rate of evaporation is slightly less than the open water rate. The turbulence introduced by the rims of the containing vessels and by irregularities in the soil surface prevents us from making a precise estimate of the ratio of the rates per unit vapour pressure gradient, but the results of other experiments agree with the implication from these that the ratio is between 0.8 and 0.9 in spite of the fact that only about one-half of the surface is available for the transmission of vapour. This is merely one aspect of a much larger problem of great biological interest in connexion with leaf transpiration and assimilation, namely, how does the rate of diffusion through a perforated plane depend upon the number, size and spacing of the perforations?

SUMMARY

Experiments on evaporation from freely drained soils are described. Under isothermal conditions characteristic *winter* field behaviour is obtained, even when the air drying power is greater than its normal English midsummer value. Characteristic *summer* field behaviour is obtained when the rapid drying of a thin surface layer is achieved, either by using an extremely high air temperature under 'isothermal' conditions, or by raising the surface temperature by means of radiation—the normal method in nature. The effect of a high salt concentration in the soil water is shown to lead to greater evaporation losses and to a tendency for the salt to concentrate in the more salty patches.

It is suggested that mulching will only be beneficial during the isothermal part of the year, i.e. when soil surface and air temperature are approximately equal, and that it will have little effect on water conservation where the soil will be self mulched by the action of summer sunshine. The cause of this self-mulching action is briefly considered in the light of our limited knowledge of soil water dynamics; it appears to

depend on the existence of a dual mechanism of water movement in soils—as liquid and as vapour—the rates of movement being very different functions of moisture content and moisture gradient.

The author wishes to express his thanks to Dr R. K. Schofield for helpful discussions on the interpretation of these experiments.

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THE RELATION OF DRAINAGE TO RAINFALL AND OTHER METEOROLOGICAL FACTORS

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(With One Text-figure)

I. INTRODUCTION

CROWTHER (1930) and KOSHAL (1934) used the Rothamsted records to obtain regression equations connecting drainage with rainfall and temperature. The former considered the period from 1878 to 1928, and the latter from 1878 to 1932.

Crowther took mean monthly values for the average of three gauges (20, 40 and 60 in. deep), and obtained their regressions on the mean monthly air temperature and on the mean monthly rainfall. He concluded that the reduction of drainage by high summer temperature was so great ($r_{DT} = -0.77$, $P < 0.01$) that it entirely masked the rainfall effect ($r_{DR} = +0.47$, $P = 0.1$). He considered that greater evaporation due to higher temperature was mainly responsible for low drainage in summer months. However, he found both partial correlation coefficients ($r_{DT.R} = -0.97$) and ($r_{DR.T} = +0.94$) highly significant.

Koshal dealt with each of the three gauges and each of the 12 months separately. He thus calculated thirty-six regression formulae on rainfall and mean air temperature. To make allowance for any slow secular changes affecting the physical condition of the soil in the gauges, he also took time as an additional independent variate. He found drainage to be closely correlated with rainfall, but for temperature he got results which seemed paradoxical. In general the coefficients were small, and non-significant, seven being positive and five negative. For May and September the coefficients were significant and positive, while for February and March they were significant and negative. The higher drainage in winter months might, he thought, be largely due to the accumulation of water during the rainy months of autumn, and the lower drainage in summer to the partial depletion of the gauges during the lower rainfall of the spring.

The complementary nature of the Crowther and Koshal treatments has been discussed by Penman & Schofield (1941; preceding paper). They reject Koshal's explanation of the apparent anomaly in the effect of temperature on evaporation, and the present work was undertaken to see how far a different method of approach would confirm the statistical findings of Koshal. Accordingly, some of the data from the 20 in. gauge have been re-examined, in the hope of elucidating the dependence of evaporation on meteorological conditions, and only the three summer months, June, July and August, have been considered. Since evaporation is likely to be greatest during these months a study of the deficits (excess of rainfall over drainage) is then most likely to show correlation with the various meteorological factors which may possibly influence evaporation. Moreover, Koshal's work has already shown that during the winter months practically the whole of the variation in drainage can be explained in terms of variation in rainfall.

II. METHOD

Periods called "natural drainage periods" were first selected from three summer months of June, July and August. A natural drainage period, as defined by Penman & Schofield, is the period between any one cessation of flow of a drain gauge and the next cessation. For example: suppose a drain gauge stops running on 1 June, starts running again on 4 June, and stops again on 7 June, then the 6 days period between 2 and 7 June will be a natural drainage period. This procedure has the advantage over the use of calendar months in that an unknown amount of evaporation will have occurred at the end of any one month after the last day on which the gauge ran. Consequently, the correlation between deficit and meteorological factors may be expected to be closer when natural periods are used than with monthly periods. Only the 20 in. drain gauge records were used as these were considered the most reliable. There is evidence that leakage has been occurring into the 40 and 60 in. gauges. The natural drainage periods chosen were from 2 to 13 days, all those occurring in the 25 years from 1915 to 1939 being taken, with the exception of a few which were rejected on account of faulty records. The meteorological factors considered were rainfall, relative humidity, previous drainage and mean daily wind velocity in miles per hour. Relative humidity is the mean of the daily readings at 9 a.m. Mean wind velocity is calculated from the anemobiograph charts for each day, by taking the mean of six 4-hourly intervals. Previous drainage is the total drainage during 21 days previous to the

period. Temperature is the mean of maximum and minimum air temperatures.

Table 1 gives the data. Data for mean daily velocity of wind were not available previous to 1923.

III. ANALYSIS OF THE DATA

The relation of the deficit to the meteorological factors was studied. As rainfall was obviously the main factor influencing both deficit and drainage, this was considered first.

Fig. 1 shows the deficit per day plotted against mean rainfall per day; the small figures indicate the number of days in the period concerned. The 45° line represents the upper limit beyond which the points cannot lie, since drainage cannot be negative.

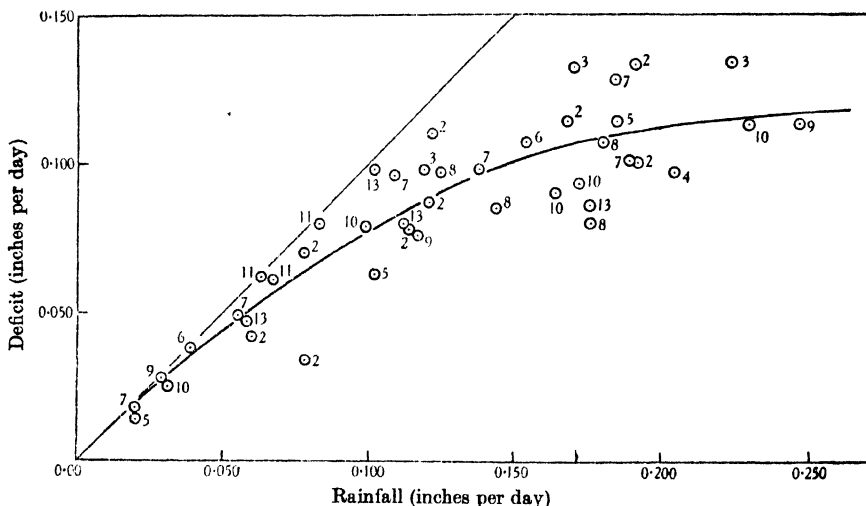


Fig. 1. Mean daily deficit and mean daily rainfall (20 in. gauge for June, July and August).

It is obvious that there is little correlation between the deficit per day and the length of the period. A cubic curve passing through the origin and having a slope of 45° at this point was, therefore, fitted to the whole of the data, with the exception of one period with a rainfall of 0.58 in. per day (3-day period), the main object of the fitting being to obtain the residuals for the study of their correlation with other meteorological factors.

Table 1

Period days	Rainfall in. per day	Drainage in. per day	Residual deficit in. per day	Relative humidity %	Mean air tem- perature ° F.	Previous drainage, total of 21 days in.	Velocity of wind miles per hour
2	0.169	0.055	+0.008	69	52	0.710	2.1
2	0.122	0.012	+0.021	71	57	0.753	7.9
2	0.078	0.008	+0.007	65	52	0.327	6.2
2	0.121	0.034	-0.001	67	54	1.104	—
2	0.114	0.036	-0.006	82	56	1.172	—
2	0.191	0.058	+0.022	83	55	0.842	—
2	0.192	0.092	-0.011	78	57	0.139	—
2	0.060	0.018	-0.009	69	53	1.302	—
2	0.078	0.044	-0.030	82	57	1.431	—
3	0.119	0.021	+0.011	66	55	0.692	2.5
3	0.223	0.089	+0.019	59	57	0.777	7.1
3	0.170	0.038	+0.026	85	58	0.513	—
3	0.580	0.371	—	80	61	—	—
4	0.204	0.107	-0.016	79	61	0.086	—
5	0.185	0.071	+0.004	80	59	1.243	—
5	0.102	0.039	-0.015	79	55	1.310	—
5	0.020	0.006	-0.005	73	61	0.628	—
6	0.154	0.047	+0.006	65	57	0.276	3.6
6	0.039	0.001	+0.002	70	59	1.656	—
7	0.138	0.040	+0.002	77	54	0.295	5.3
7	0.020	0.002	-0.001	85	58	0.974	4.7
7	0.109	0.013	+0.014	73	56	1.010	—
7	0.184	0.056	+0.018	84	63	0.360	—
7	0.189	0.088	-0.010	81	58	1.545	—
7	0.055	0.006	+0.001	79	57	1.801	—
8	0.125	0.028	+0.007	84	61	1.443	6.0
8	0.144	0.059	-0.013	87	67	0.712	0.96
8	0.176	0.096	-0.028	78	59	0.120	5.8
8	0.180	0.073	-0.002	84	56	0.651	—
9	0.246	0.133	-0.003	81	57	0.045	—
9	0.029	0.001	+0.001	82	56	0.323	—
9	0.117	0.041	-0.010	81	55	0.365	—
10	0.164	0.074	-0.014	84	58	0.699	3.0
10	0.099	0.020	+0.002	78	59	0.089	5.2
10	0.229	0.116	-0.002	65	64	0.003	4.2
10	0.172	0.079	-0.014	76	56	0.751	—
10	0.031	0.036	-0.004	81	62	2.562	—
11	0.063	0.001	+0.009	81	64	0.001	1.9
11	0.083	0.003	+0.013	78	65	0.053	—
11	0.068	0.007	+0.004	87	60	0.260	—
13	0.058	0.011	-0.003	85	64	0.640	1.9
13	0.176	0.090	-0.022	86	59	1.227	—
13	0.102	0.004	+0.019	75	59	0.776	—
13	0.112	0.032	-0.004	79	58	0.189	—

The equation of the curve must be of the form

$$R - D = R - aR^2 - bR^3,$$

where a and b are given by

$$a\Sigma R^4 + b\Sigma R^5 = \Sigma DR^2, \quad a\Sigma R^5 + b\Sigma R^6 = \Sigma DR^3.$$

Solving these equations, we obtain

$$a = 2.3277, \quad b = -0.6958.$$

Thus the relationship between rainfall and deficit is given by

$$R - D = R - 2.3277R^2 + 0.6958R^3.$$

The sum of the squares of the residuals (41 D.F.) was found to be 0.007190, giving a mean square of 0.000175 and a standard deviation of ± 0.0132 .

The correlation of these residuals with relative humidity and mean air temperature was next investigated. The regressions were found to be

On relative humidity: -0.00048 ± 0.00029 .

On air temperature: -0.00009 ± 0.00059 .

The regression on relative humidity, though not fully significant, is of the expected sign. There is no evidence of any influence of air temperature.

The deficit also shows a slight correlation with mean wind velocity, the regression of the residuals on wind velocity being $+0.00217 \pm 0.00176$. This, though not significant, is also in the expected direction.

In an attempt to obtain explicit evidence for Koshal's hypothesis of accumulation, the regression of the residuals on drainage during the 3 weeks previous to the natural periods was also calculated. The coefficient -0.00287 ± 0.00361 does not approach significance, though it has the sign to be expected from Koshal's hypothesis.

IV. DISCUSSION

The device of considering natural drainage periods has been successful in accounting for most of the variation in deficit in terms of rainfall. The residual standard deviation of ± 0.0132 in. per day is slightly less than the residual standard deviation obtained by Koshal (for the monthly periods of June, July and August) of ± 0.0144 in. per day, in spite of the fact that the periods taken are much shorter (2-13 days).

The curvilinear relationship between deficit and rainfall indicates that for small amounts of rainfall most of the rainfall is lost, but that as the rainfall increases the loss per day approaches an upper limit. The length of period appears to have little influence on the rate of loss.

Attempts to relate the residual deficit (after allowing for rainfall) with relative humidity, temperature and wind showed slight (though not significant) correlations in the expected directions with humidity and wind but none with mean air temperature. The temperature results thus confirm Koshal's conclusion that there is little association of deficit with mean air temperature. This, of course, does not prove that the

deficit is not largely or wholly due to evaporation. The amount of evaporation must depend on the moisture content of the surface layers of the soil, and it is quite probable that the variations in evaporation rate produced by variations in other meteorological factors are largely masked by the fact that there is only a limited amount of water that can be easily evaporated. The form of the rainfall curve is also that which we should expect to obtain if the deficit were entirely due to evaporation.

SUMMARY

Natural drainage periods, i.e. periods between consecutive cessations of flow of the Rothamsted 20 in. drain gauge, were selected for the months of June, July and August. All periods of from 2 to 13 days were taken.

A curvilinear relationship between the difference of rainfall and drainage (deficit) and rainfall was established.

Residuals from this curve showed no appreciable correlation with the mean air temperature, but there was some slight evidence that the deficit was increased by a decrease in relative humidity or an increase in wind velocity.

The residuals showed little correlation with drainage during the previous 3 weeks.

My acknowledgements are due to Dr Yates, Dr Schofield, Dr Penman and Mr Boyd for their advice and assistance.

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THE MINERALS IN THE CLAY FRACTIONS OF A BLACK COTTON SOIL AND A RED EARTH FROM HYDERABAD, DECCAN STATE, INDIA

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(With Plate XII and Two Text-figures)

METHODS for determining the mineral constituents of soil colloids have recently been discussed by Nagelschmidt (1939). These methods were applied to soil samples from a typical black cotton soil (or Regur) and a typical red earth from Hyderabad, Deccan State, India, which have been studied in detail by Desai (1939).

The two soil profiles selected for this study were taken on and near the Government Experimental Farm, Rudrur, Nizamabad. The climate of the district is characterized by cool, dry winters, a rainy season which extends from June to October and a summer season, February to June, during which severe local storms may occur. The total rainfall ranges between 37 and 40 in. per annum.

The black cotton soil (no. P7 in Desai, 1939) was taken from an irrigated area on the farm. It was derived from colluvium over gneiss and consisted of a dark greyish heavy clay loam which was hard and compact to the lowest depth of sampling, 48 in. The fine material contained little calcium carbonate, but some nodules and concretions were distributed throughout the profile; there was no pronounced zone of carbonate accumulation. Its pH values increased with depth from 8.0 to 8.4, and the soluble salt content varied between 0.05 and 0.07 %.

The red earth (no. P8 in Desai, 1939) was taken on uncultivated waste land on the slope of a granite hill near the farm. It was a brownish red coarse sandy clay loam, becoming lighter in colour with depth, and containing disintegrating rock material in the lowest sample, 36–42 in. The pH values increased with depth from 6.1 to 7.4, no carbonates being

present. The soluble salts varied between 0.04 and 0.06%, except for one high value 0.09% at 6-18 in.

MECHANICAL ANALYSIS

The samples of the black cotton soil contained about 5% stones, larger than 2 mm. These were mainly greyish carbonate nodules with a few rock fragments of weathered granite similar to those found in the red soil, and some pieces of vein quartz. The soils of the red earth, P8, had far more (30-40%) stones consisting entirely of weathered fragments of coarsely crystalline granite or gneiss.

Mechanical analyses for six samples of each profile are given in Table I. The data show that there is very little variation within each profile. The black soil has 60-70% silt and clay, whereas the red soil has only 25-40%.

Table I. *Mechanical analyses: oven-dry fractions as percentage of oven-dry soil below 2 mm.*

Depth in.	Coarse sand	Fine sand	Silt	Clay	Air-dry moisture
Black cotton soil (P7)					
0-6	23.0	13.2	22.6	39.9	6.6
6-12	21.8	14.0	22.8	41.1	6.6
12-18	23.2	13.7	23.6	39.7	6.2
18-24	22.2	12.6	22.8	41.7	6.6
30-36	21.4	12.4	23.2	43.3	6.8
42-48	20.1	11.9	23.1	44.7	6.9
Red earth (P8)					
0-6	52.2	23.0	5.9	20.0	2.5
6-18	46.4	15.9	6.5	31.8	4.2
18-24	48.8	13.5	7.8	32.0	4.4
24-30	51.7	15.0	7.9	26.8	3.8
30-36	48.0	15.3	9.0	29.0	4.1
36-42	47.0	13.8	8.7	31.3	4.5

Clay fractions were prepared by treating the soils with hydrogen peroxide and acetic acid, washing till the filtrates were free from calcium, and dispersing with ammonia. The clay was separated by repeated decantations at 8.5 cm. after 24 hr.

The clay suspension was subdivided into three fractions, coarse, fine and superfine clay, by repeated supercentrifuging at 23,000 rev./min. with rates of flow of 120 l./hr. and 12 l./hr. The estimated particle sizes of the three fractions were:

Coarse clay	1.4-0.1 μ diameter
Fine clay	0.1-0.06 μ diameter
Superfine clay	Under 0.06 μ diameter

Quantitative results for two representative samples of each profile are shown in Table II. In both profiles the coarse clay forms the smallest and the superfine clay the largest fraction. The proportions of coarse, fine and superfine clay are constant throughout the black profile, but for the red profile the superfine clay increases with depth at the expense of the fine one.

Table II. *Clay subfractions as percentages of total clay*

Soil	Depth in.	Coarse clay	Fine clay	Superfine clay
Black	12-18	10	25	65
Black	42-48	11	22	67
Red	18-24	7.5	43.5	49
Red	36-42	8.5	27.5	64

CHEMICAL ANALYSIS OF CLAY SUBFRACTIONS

Silicate analyses were carried out on all fractions obtained from the two soil profiles. The results show that in each case there is very little variation with increasing depth. As the full data are being published elsewhere (Desai, 1939), it seems sufficient to give results only for one layer from each profile. The data are shown in Table III and include the base exchange capacities determined by the Parker (1929) method, silica-alumina and silica-sesquioxide ratios and the organic carbon determined by the modified chromic acid method (Robertson & Shewan, 1935).

Table III. *Chemical analyses of clay subfractions for one black cotton soil (P7) and one red earth (P8), as percentages of oven-dry clays*

	Black soil, 12-18 in.			Red earth, 18-24 in.		
	Coarse %	Fine %	Superfine %	Coarse %	Fine %	Superfine %
SiO ₂	57.30	47.70	49.40	46.39	42.07	45.43
Al ₂ O ₃	16.00	23.00	22.80	25.04	26.01	26.37
Fe ₂ O ₃	10.60	11.90	12.00	11.79	15.48	10.91
TiO ₂	2.00	1.39	0.30	1.54	0.94	0.22
MnO	0.05	0.05	0.04	0.05	0.05	0.04
CaO	0.58	0.22	Nil	0.49	0.48	0.53
MgO	2.38	2.30	1.95	1.08	1.03	1.02
Na ₂ O	0.77	0.39	0.28	0.55	0.55	0.28
K ₂ O	1.71	1.35	0.71	2.13	1.00	0.67
P ₂ O ₅	0.10	0.14	0.11	0.17	0.24	0.21
Ignition loss	9.04	12.30	13.70	10.93	13.09	14.68
Total	100.53	100.74	101.29	100.16	100.94	100.36
SiO ₂ /Al ₂ O ₃	6.1	3.5	3.7	3.2	2.8	2.9
SiO ₂ /R ₂ O ₃	4.3	2.7	2.8	2.4	2.0	2.3
Exchange capacity in mg. equivalent per 100 g. clay	34	75	99	23	36	49
Organic carbon	—	2.08	2.44	—	1.65	1.70

DETERMINATION OF FREE OXIDES

Free silica, alumina and iron oxide were determined by the hydrogen sulphide method of Drosdoff & Truog (1935). The amounts dissolved and the base exchange capacity before and after the treatment were determined on one unfractionated clay sample of each profile and on one fine and superfine clay sample of the red earth. The results are shown in Table IV. A number of samples were subjected to the modified Truog treatment using sodium sulphide and oxalic acid (Truog *et al.* 1937). This method is more drastic and the total loss of material is higher, 40% of the superfine red fraction being dissolved as against 10% with hydrogen sulphide. It is likely that in this process the silicates present are attacked to some extent (cp. Raychaudhuri, 1936; Toth, 1939).

Table IV. *Alumina, iron oxide and silica, as percentage of clay, dissolved by treatment with hydrogen sulphide according to Drosdoff & Truog (1935), and base exchange capacities in mg. equiv. per 100 g. clay before and after the treatment*

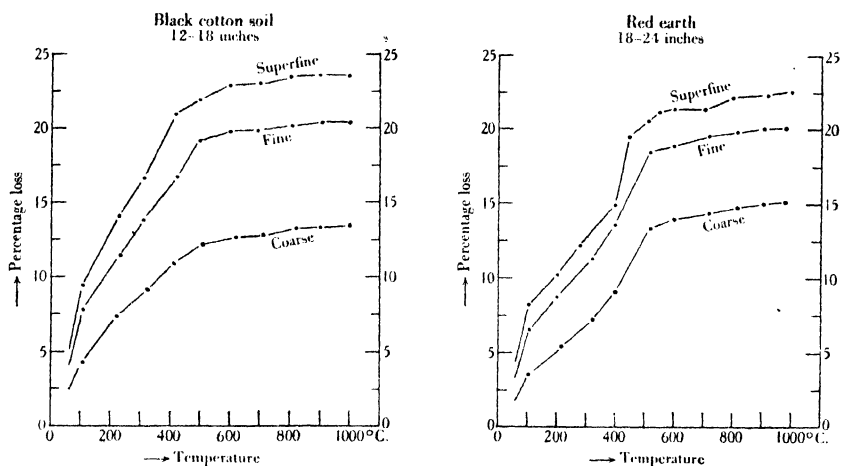
	Black whole clay 42-48 in.	Red whole clay 24-30 in.	Red fine clay 24-30 in.	Red superfine clay 24-30 in.
SiO ₂	2.30	3.98	2.37	2.62
Al ₂ O ₃	0.56	1.10	0.57	1.29
Fe ₂ O ₃	4.85	6.98	13.40	5.70
Base exchange capacity:				
Before treatment	83	35	—	—
After treatment	58	30	—	—

DEHYDRATION DATA

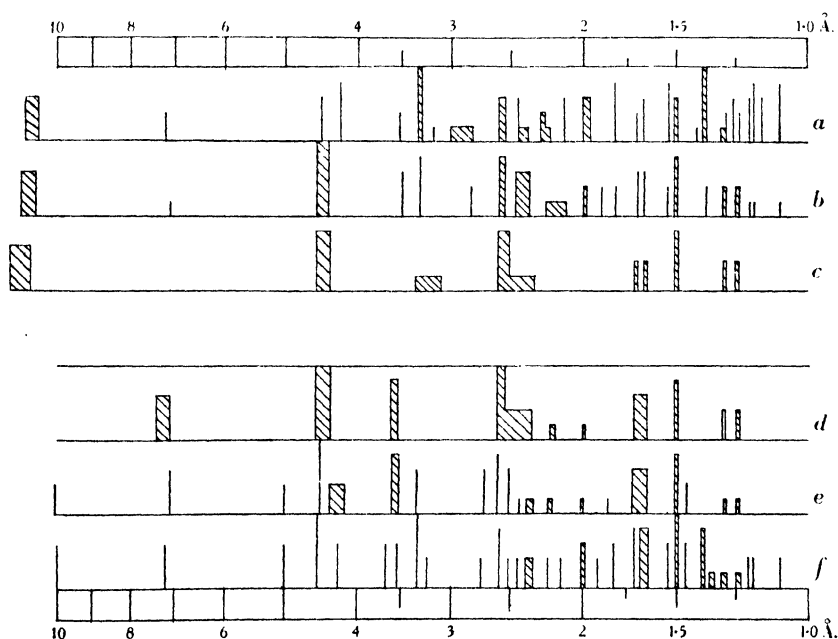
Dehydration curves were taken for three depths in each profile by the method of intermittent heating. Again, there was little or no variation with depth for each profile. Characteristic curves are shown in Text-fig. 1. The water lost at 105° increased rapidly with decreasing grain size for all fractions, and was consistently about 1% higher for fractions from black soils than for the corresponding red ones. The pronounced difference in the form of the curves for red and black soils, especially between 400 and 600°, is discussed in detail later (pp. 647, 650).

X-RAY DATA

Powder and aggregate diagrams were taken for most of the samples and it was found that throughout each profile the clay fractions of equal grain size gave identical diagrams. There were, however, differences between the diagrams from the two profiles and also between the coarse,



Text-fig. 1. Dehydration curves of clay subfractions from black cotton soil and red earth, by the method of intermittent heating. Loss in weight as percentage of air-dry fractions.



Text-fig. 2. X-ray data for black cotton soil (a) coarse, (b) fine, (c) superfine clay and red earth, (d)-superfine, (e) fine, (f) coarse clay.

fine and superfine fractions of any one clay. This confirms the chemical data and shows that there is no noticeable variation in the mineralogical composition throughout each profile.

Text-fig. 2 shows the diffraction data in the form described previously (Nagelschmidt, 1939), and Pl. XII shows some of the diagrams. Diagrams were also taken for samples after treatment by the sodium sulphide-oxalic acid method (Truog *et al.* 1937). For all black clays there was no difference between treated and untreated samples, but for most of the red clay samples the diagrams of the treated material contained fewer lines, and, except for one line, the difference could be explained as due to the disappearance of hematite, $\alpha\text{-Fe}_2\text{O}_3$. The one line not due to hematite coincided with one of the strong lines of goethite, $\alpha\text{-FeOOH}$, which may have been present in very small amounts. There was no indication of the presence of crystalline aluminium hydroxides.

Diagrams of samples of the black clay at different moisture contents showed the lattice shrinkage and expansion characteristic of a member of the montmorillonite group. The red clays failed to show this effect under the same conditions. Aggregate diagrams of the air-dry black clay showed the basal spacing to be about 12 Å.; the corresponding diffraction line was blurred and not very strong. By the method of separation the exchangeable ions of this clay were ammonium and hydrogen, but after the material had been leached with calcium chloride and the excess chloride washed out, new air-dry aggregates showed a stronger basal spacing of 15 Å. The replacement of ammonium and hydrogen by calcium would explain this change in the basal spacing at constant relative humidity. Similar results for supercentrifuged bentonite fractions have been reported previously (Nagelschmidt, 1939). The basal reflexions of the soil colloids were, however, less intense than those of the bentonite. The interpretation of the X-ray diagrams is given in Table V.

Table V. *Minerals identified by X-ray diffraction in clay subfractions*

	Black cotton soil, 12-18 in.			Red earth, 18-24 in.		
	Coarse	Fine	Superfine	Coarse	Fine	Superfine
Quartz	Much	Little	Nil	Medium	Trace	Nil
Mica	Trace	Trace	Nil	Little	Little	Nil
Kaolinite or halloysite	Little	Little	Nil	Much	Much	Much
Montmorillonite or beidellite	Medium	Much	Much	Nil	Nil	Little?
Hematite and goethite	Nil	Nil	Nil	Little	Little	Trace

OPTICAL DATA

A petrological examination of the sand fractions from one sample of each profile was carried out by Dr R. Hart, to whom our thanks are due for the following information. "The black soil contained mainly quartz and a few grains of feldspar, hornblende and some iron oxide, while the red soil contained the same minerals and, in addition, some brown mica. There was much more feldspar and ferromagnesian minerals in the red soil than in the black one, and the quartz was much more heavily stained by iron oxides."

The refractive indices of some of the superfine clay samples were measured in sodium light with a mixture of olive oil, cinnamon oil and bromo naphthalene, as recommended by Correns & Mehmel (1936). The samples were saturated with calcium and measured after they had reached equilibrium weight, at room temperature at 50% relative humidity, and again after drying at 105° C., both before and after sodium sulphide-oxalic acid treatment (Truog *et al.* 1937). The results for n_γ are given in Table VI. The double refraction of the black samples of the order of 0.013 was not noticeably affected by the drying or by the Truog treatment, and the double refraction of the red samples was not affected by the drying; it was, however, reduced by the Truog treatment from 0.017 to 0.010.

Table VI. *Refractive index n_γ determined in sodium light for superfine clay fractions before and after Truog treatment and at two temperatures*

	Black cotton soil, 12-18 in.		Red earth, 18-24 in.	
	Superfine	Truog-treated superfine	Superfine	Truog-treated superfine
Drying				
50% R.H.	1.579	1.577	1.588	1.581
Oven-dry	1.592	1.588	1.600	1.585

CORRELATION OF RESULTS

Although a general knowledge of the minerals present in a soil colloid can be obtained by adequate X-ray diffraction data alone, this knowledge is made more certain and can be refined by using chemical, dehydration and optical data. In this way quantitative statements can be made and the fitting together of the various observations checked. We shall first consider the clay of the black cotton soil (12-18 in.) and then that of the red earth (18-24 in.), in both cases beginning with the superfine fractions.

BLACK CLAY SUPERFINE FRACTION

This fraction shows from the X-ray data only one crystallized mineral, a member of the montmorillonite group. The observed base exchange capacity of 99 mg. equivalent per 100 g. clay is in good agreement with this observation. Its composition (Table III) closely resembles that of beidellite from Beidell, Saguache Co., Colorado (Larsen & Wherry, 1917), as can be seen by comparing the percentages for silica, alumina and iron oxide for beidellite and the superfine fraction, calculated in both cases for ignited material.

	Beidellite from Beidell %	Black superfine clay %
SiO ₂	58.6	57.7
Al ₂ O ₃	25.1	26.6
Fe ₂ O ₃	10.8	14.0

In order to see whether appreciable amounts of amorphous material were present in the soil colloid, hydrogen sulphide treatments (Drosdoff & Truog, 1935) were given to the superfine black clay and also to the beidellite from Beidell. The amounts of silica, iron oxide and alumina dissolved were as percentages of the air-dry material.

	Beidellite from Beidell %	Black superfine clay %
SiO ₂	3.59	3.02
Al ₂ O ₃	0.23	0.14
Fe ₂ O ₃	5.64	4.06

Both materials were slightly attacked by this treatment, but there was no evidence for the presence of large amounts of amorphous material in the superfine black clay.

A method of calculating isomorphous replacements and base exchange capacities for members of the montmorillonite group has been described by Nagelschmidt (1938). By this method the superfine black clay contains in the silicon layer Si_{3.49} Al_{0.51} instead of Si₄ and in the aluminium layer Al_{1.38} Fe_{0.62}, no divalent ions being present, instead of Al₂. The calculated base exchange capacity is 120 mg. equivalent per 100 g. oven-dry material which is somewhat in excess of the observed value of 99 mg. equivalent per 100 g.

Grim (1939) has recently suggested that the mica type material (illite) has negative charges due to isomorphous replacements mainly in the silicon layers, whereas the montmorillonite type with variable basal

spacing has these replacements mainly in the aluminium layer. The observations on the black superfine colloid do not seem to bear this out, as there is a variable basal spacing and the replacements are mainly in the silicon layers. It can be argued, however, that the iron oxide determined may include an unknown amount of divalent iron.

The dehydration curve of the superfine black clay differs from known montmorillonite or bentonite dehydration curves in being almost straight up to about 500° , whereas in montmorillonite little water is lost between 300 and 450° . Unfortunately, through lack of material, we were unable to take a dehydration curve of beidellite. It is possible that the iron in beidellite causes its dehydration curve to be different from that for montmorillonite.

The refractive index of the superfine black clay at 105° , $n_D = 1.592$, is very similar to the refractive index of beidellite from Beidell, taken at 105° , $n_D = 1.589$, but the air-dry values are somewhat different. This deviation may be due to differences in water content and exchangeable ions.

BLACK CLAY FINE FRACTION

This fraction contains, according to the X-ray data, much montmorillonite, little quartz and kaolinite and very little mica. The estimation of kaolinite and mica is mainly based on the intensities of basal reflexions on aggregate diagrams, the estimation of quartz on reflexions at larger angles of diffraction.

In order to determine the amount of kaolinite present the dehydration curve is usually of great help, as kaolinite loses more than 10% of water between 350 and 500° , whereas montmorillonite or mica lose only 2–3% in this interval. The curve of the superfine black clay is, however, different from montmorillonite curves; it is, therefore, impossible to estimate the kaolinite from these curves. It seems, however, permissible to assume as a first approximation that the base exchange capacity and chemical composition of the Beidellite, which was identified as the only constituent of the superfine fraction, are constant from superfine to coarse clay. With this assumption 75% of beidellite would be a maximum estimate for the fine fraction if the other minerals present had no base exchange capacity at all. Actually the base exchange capacity of kaolinite is low, and could hardly account for more than 10 mg. equivalent %, and mica is only present in very small amounts. 60% of beidellite would therefore seem a minimum and 70% the most likely value. If we now subtract the equivalent of 70% of the superfine fraction from the fine fraction, using

the chemical data in Table III, we are left with a residue of 13.1% SiO_2 and 7.1% Al_2O_3 , both as percentage of the fine fraction. The aluminium is mainly present in kaolinite and a small proportion in mica. It is therefore possible to state that 17% kaolinite and 5% quartz are maximum estimates under the above assumptions. Actually both percentages should be somewhat smaller, as there is some mica present.

The mica in soil colloids may vary widely in chemical composition, but in comparable materials the potassium content is probably the best guide. The lowest potassium content recorded by Grim *et al.* (1937) for illite is 4.7% K_2O , but earlier data by Denison *et al.* (1929) seem to indicate that much lower amounts, down to less than 1%, can occur in coarse mica in soils. The K_2O content of the fine black clay is 0.64% larger than the K_2O content of the superfine fraction. On the basis of 5% K_2O for the mica in soil colloids in general this would correspond to 13% of mica in the fine fraction. As it seems impossible to isolate the mica from the black clay, there is no proof that this figure is correct, but higher values seem very unlikely from the X-ray data, especially from the intensity of the basal 10 Å reflexion. Recently, Hendricks & Alexander (1939) have suggested that minerals with mixed structures between hydromica and montmorillonite may frequently occur in soil colloids, and although no details about such minerals are known, it seems likely that their basal reflexions would be blurred and weaker than those of the pure minerals; in this way estimates based on X-ray data alone would tend to be too low in comparison with estimates for layer-lattice minerals such as kaolinite, which do not form such mixed structures. For the various reasons given above, it is not possible to determine the various constituents with great accuracy, but the following are the extreme amounts which may be present: beidellite 60–75%, kaolinite 5–20%, mica 5–20%, quartz 2–10%. The most likely composition is beidellite 70%, kaolinite 10%, mica 15%, quartz 5%.

BLACK CLAY COARSE FRACTION

This fraction contains according to the X-ray data much quartz, medium beidellite, little kaolinite and a trace of mica. If the considerations elaborated above for the fine fraction are applied, it is seen that the limiting values for the different constituents are: quartz 25–32%, beidellite 25–35%, kaolinite 10–23%, mica 5–25%. The most likely values are: beidellite 30%, quartz 30%, kaolinite 15%, mica 15%. The analytical data also show that there is too much iron present to be

accommodated in beidellite and mica on the assumption that the beidellite has the composition of the superfine fraction. It seems that 4% Fe_2O_3 is present as amorphous or crystalline hydroxide or oxide. As amounts less than 5% of goethite in mixtures with other soil colloid minerals can probably not be detected by X-ray diagrams, this result is consistent with the X-ray data.

The total water content of the coarse fraction is 66% of the water content of the superfine fraction, and if we take all minerals other than quartz together as being hydrated, we get a rough estimate for quartz of 34%, which does not deviate much from the composite estimate of 30% given above.

RED CLAY SUPERFINE FRACTION

According to the X-ray data, this fraction contains much kaolinite or halloysite, and only a trace of iron oxide or hydroxide. If we assume that all aluminium shown in the analysis of this fraction in Table III represents a kaolinite of theoretical composition, we get a value of 67% kaolinite and are left with a residue of 14.5% SiO_2 and 10.9% Fe_2O_3 . The SiO_2 cannot be quartz, as such amounts of quartz would show up on the X-ray diagrams, but it also cannot be amorphous silica, as the Truog treatment dissolves less than 3% of SiO_2 , and amorphous silica should be dissolved by that treatment. The presence of a hypothetical iron kaolinite, which would account for the excess silica, seems very unlikely, although it cannot at the present stage of our knowledge be definitely ruled out for soil colloids. It was decided, therefore, to use a sodium sulphide-oxalic acid treatment to remove iron oxides, followed by prolonged heating at 510° to destroy kaolinite. This treatment should destroy all crystalline materials recognized in the original X-ray data. The X-ray diagrams of the treated material showed a number of weak lines corresponding to hk0 reflexions of montmorillonite or beidellite, but no lines corresponding to their basal reflexions. Aggregate diagrams of the heated material showed no clear basal reflexions.

Aggregates of the calcium-saturated original air-dry superfine fraction showed, besides strong kaolinite basal reflexions, only a doubtful trace of a 15 A. line. The presence of beidellite in this fraction is therefore possible but not quite certain. It would account for part of the base exchange capacity of 49 mg. equivalent per 100 g. clay, which is higher than would be expected for kaolinite, and for part of the loss of water below 300°C. , which is also higher than would be expected for kaolinite or halloysite

in the presence of less than 3% organic matter. The properties of this fraction seem to correspond closely to the data given by Kelley *et al.* (1939) for the Vina colloids.

The dehydration curve of this fraction shows that the bulk of the lattice hydroxyl of the kaolinite is given up below 450° C., which would, according to the dehydration curves of Ross & Kerr (1934), indicate halloysite rather than kaolinite, but it has been shown previously (Nagelschmidt, 1939) that these two minerals cannot be distinguished in soil colloids. The refractive index, even after the sodium sulphide-oxalic acid treatment (Truog *et al.* 1937) is higher than would be expected for kaolinite.

On summarizing our evidence it can only be said that about 60% of the fraction consists of kaolinite or halloysite and less than 10% of iron oxide and hydroxide. Up to 30% of it may be due to a member of the montmorillonite group.

RED CLAY FINE FRACTION

According to the X-ray data, this fraction also contains much kaolinite or halloysite, and in addition a little hematite, mica and a trace of quartz. From the chemical and dehydration data kaolinite forms 50–60% of the fraction. The amount of Fe_2O_3 dissolved by the hydrogen-sulphide treatment (Drosdoff & Truog, 1935) is 10%, which gives an upper limit for free iron oxide, the remaining iron being partly in the mica and partly perhaps in a member of the montmorillonite group. The quartz content is less than 5%, this estimate being based on the X-ray diffraction data. It again seems that there is slightly more SiO_2 shown in the analysis than can be accounted for as kaolinite, quartz and amorphous silica, but the excess is less than in the superfine fraction. Diffraction diagrams of material after sodium sulphide-oxalic acid treatment and heating to 510° C. showed some quartz and mica lines. In the presence of these lines it is impossible to see whether or not any of the hk0 lines of montmorillonite, found on corresponding diagrams of the superfine fraction, are present. If this mineral is present, the base exchange capacity, dehydration and chemical data show that its amount is less than that in the superfine fraction. The composition of this fraction seems to be 50% kaolinite, 15% mica, 10% oxide and hydroxide of iron, 3% of quartz and possibly up to 15% of a member of the montmorillonite group.

RED CLAY COARSE FRACTION

This fraction differs from the fine clay only in having more quartz and slightly more mica. An estimate based on the principles outlined above gives kaolin or halloysite 40%, mica 30%, quartz 10%, iron oxide and hydroxide 5–10%. There is no reason to assume the presence of a member of the montmorillonite group in this fraction.

The results of the estimates for all six fractions are summarized in Table VII.

Table VII. *Minerals present in fractions of clay as percentage of fraction*

	Black cotton soil, 12–18 in.			Red earth, 18–24 in.		
	Coarse	Fine	Superfine	Coarse	Fine	Superfine
Beidellite	30	70	90	—	(?) 15	(?) 30
Kaolinite	15	10	—	40	50	60
Mica	15	15	—	30	15	—
Quartz	30	5	—	10	3	—
Hematite and goethite	5	—	—	8	10	10

DISCUSSION

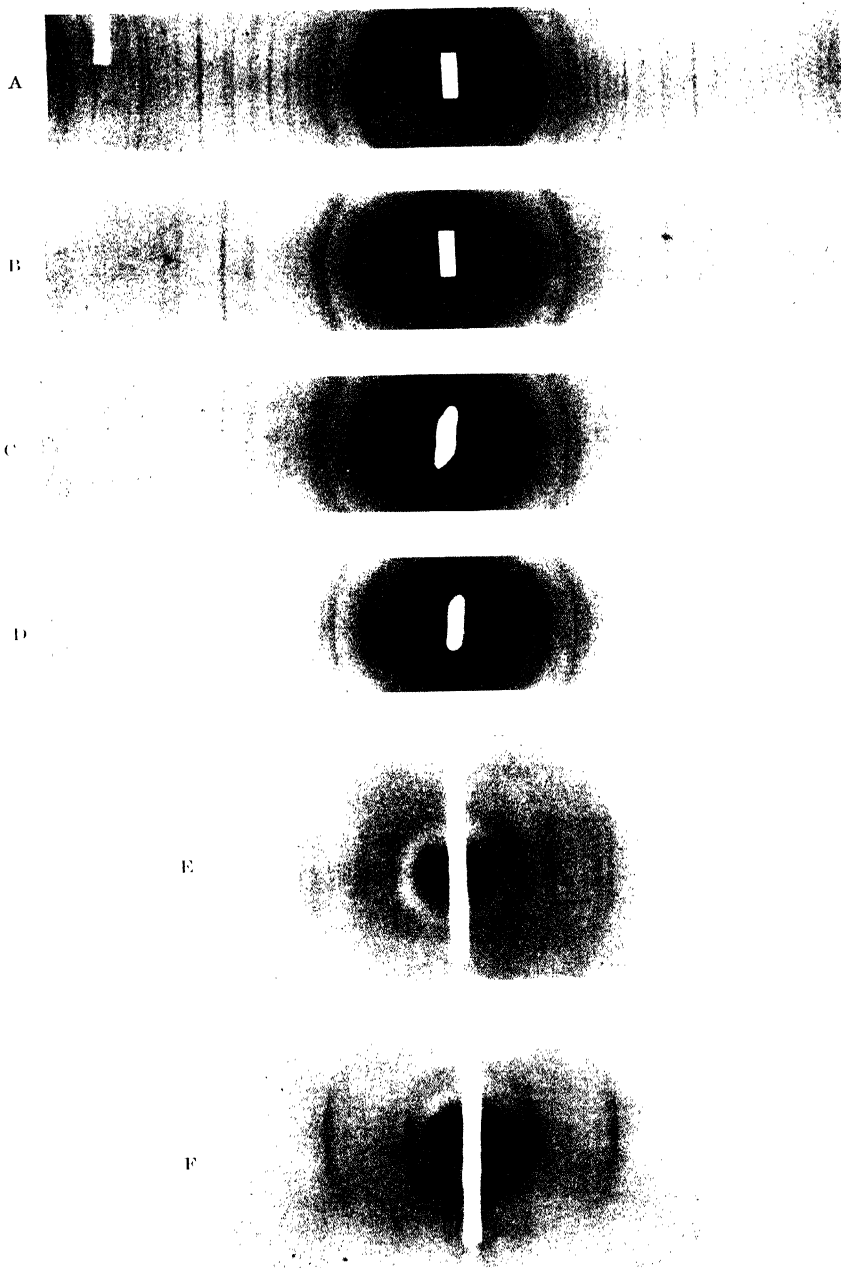
The red and the black soils under investigation are derived from the same or from very similar rocks, and as they occur near each other they are exposed to the same climatic conditions. The differences in the mineralogical compositions of the two clays must therefore be due to processes resulting from the difference in the topographical position of the two soils, the red soil occurring on a slope of waste land, while the black soil is found in the plain in an irrigated area. According to Desai (1939) it is a general experience that red soils in the vicinity of black cotton soils are only found on slopes. There are many indications showing that the red soil is eluvial, being strongly leached, whereas the black soil is illuvial, receiving leaching products. The percentage of particles above 2 mm. is very high in the red soil, and the silt and clay contents are comparatively low. Both conditions are reversed in the black soil, and a large proportion of its particles above 2 mm. consist of secondary formations, carbonate concretions. Relatively high proportions of feldspar and hornblende are found in the fine sand fractions of the red soil, but these minerals have almost disappeared from the black soil. The red soil has a lower pH than the black one, especially in the top layer. The data given in this study show that under such eluvial conditions kaolinite or halloysite is the main constituent of the clay, whereas in the illuvial

black soil a member of the montmorillonite group predominates in the clay. Correns & Engelhart (1938) have shown that the weathering of felspar is a molecular process and that the rate at which alumina and silica are dissolved depends within certain limits on the acidity of the leaching solution. Which secondary minerals are formed, and where they are formed in nature will largely depend on the conditions of drainage and on the type and amount of other ions, mainly alkalies and alkaline earths, present.

Kaolin and montmorillonite have been synthesized in hydrothermal bomb experiments and the conditions of their formation investigated. According to Noll (1936) the amount and type of alkalies and alkaline earths present and the pH are of greater importance than the relative amounts of silica and alumina, and under appropriate conditions both minerals are formed together. Kaolin formation is favoured by acid or neutral conditions and montmorillonite formation by alkaline conditions. Although hydrothermal bomb experiments are not directly comparable with surface weathering and the formation of clay minerals, it is noteworthy that the montmorillonite and kaolinite occur in soils of types investigated here under just those conditions of reaction under which they have been produced in the laboratory. The simplest hypothesis is that in nature they are produced in a similar manner.

It has been shown in the description of the superfine red clay that kaolin is not the only clay mineral present and that there was possibly 20–30% of a material which may be beidellite or perhaps a silicate not known as a mineral, capable of forming beidellite. This mineral decreases in amount as the particles become larger. Such material would, on account of its small size, be easily transported and might form beidellite under the conditions prevailing in the black soil.

Mica in the red clay decreases rapidly with decreasing grain size, probably indicating its instability under the conditions prevailing in the red soil. In the black soil it is absent from the superfine fraction, but occurs in about equal proportions in the fine and coarse clay. The quartz percentage of the coarse black clay is quite high, but it is impossible to say whether this quartz is residual or newly formed. There is no direct microscopic evidence for secondary quartz in the coarse clay or in the silt fraction of the black soil, which consists almost entirely of quartz. Kaolin occurs in minor amounts in the coarse and fine black clay, but not in the superfine fraction. It seems likely that this kaolin was transported from the red soil and not formed in the black soil, as in the latter case it would probably not decrease in amount with decreasing grain size.



X-ray diffraction diagrams of clay fractions. A to D, powder diagrams, E and F aggregate diagrams

- | | |
|---------------------------------------|----------------------------------|
| A. Black cotton soil, coarse clay. | E. Black cotton soil, fine clay. |
| B. Black cotton soil, superfine clay. | F. Red earth, fine clay. |
| C. Red earth, superfine clay. | |
| D. Red earth, coarse clay. | |

(For reproduction the blackening in the centre has been reduced by partial shading.)

SUMMARY

The mineral compositions of the clays from a red earth and a black cotton soil from Hyderabad, Deccan State, India, occurring in close proximity in the field are determined. Both soils are derived from the same or from very similar parent rocks, a coarsely crystalline granite or gneiss.

For both soils there is practically no variation in the mineralogical composition of the clay throughout the profile, but for any given clay there is some variation with grain size. The main contrast between the two is that the red clay contains predominantly kaolinite or halloysite, whereas the black clay contains mainly beidellite, a member of the montmorillonite group. The topography appears to be the principal factor associated with this difference in minerals, and the processes of weathering believed to have produced the contrasted clays are discussed with reference to experiments on the leaching of felspar in the laboratory and on hydrothermal synthesis.

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AEROBIC DENITRIFICATION

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(With 8 Text-figures)

DENITRIFICATION is the reduction of nitrates to gaseous nitrogen, sometimes mixed with oxides of nitrogen: it is the only process which certainly causes a loss of nitrogen in nature, because the nitrogen gas escapes into the atmosphere. It is known that nitrite certainly, and probably hyponitrite, are formed as intermediates in the course of the reaction; it is obvious that nitrate (or nitrite) must be present if denitrification is to take place; and it is also known that a compound to act as a hydrogen donor is necessary for the process. Karlsen (1938) published a detailed study of the effect of pH and of iron on denitrification by *Pseudomonas aeruginosa*; and Elema (1932) and Elema *et al.* (1934) studied the changes in oxidation-reduction potential during denitrification by *Micrococcus denitrificans*. Elema's paper includes a set of equations which represents the most probable course of the reaction.

Several details of the process remain obscure, among them being the effect of aeration. The generally accepted view is that denitrification is an essentially anaerobic process, and that it is diminished or stopped by the admission of air. This supposition is probably accepted mainly because it provides a neat teleological explanation for the fact of denitrification; oxygen is made available by the reduction of nitrate; so under anaerobic conditions the bacteria reduce the nitrate in order to supply themselves with oxygen. Under aerobic conditions they need not reduce nitrate to obtain oxygen, and it is therefore assumed that they do not reduce it. This supposition is based on very little experimental evidence; though Weissenberg (1902) described a strain of *Pseudomonas aeruginosa*, which grew on nitrite under aerobic conditions, but did not reduce it, while in anaerobic cultures the nitrite was all reduced with much gas production. There is no reason to suppose, however, that every denitrifying species is affected by aeration in the same way. Seiser & Walz (1925) found that nitrogen was lost from both aerobic and anaerobic cultures of *Ps. putida*. The effect of aeration on *Ps. denitrofluorescens* was studied by Korochkina (1936), who remarks "a relatively high pH value does not exclude denitrification. Therefore it is very difficult to eliminate denitrification by means of increased aeration."

The experiments described in the present paper were mainly concerned with the effect of aeration on denitrification; they were performed with pure cultures of two denitrifying species in simple synthetic liquid media.

SOURCE AND DESCRIPTION OF BACTERIA

The two species of denitrifying bacteria used in this work are classifiable under the genus *Pseudomonas*, as defined by Dowson (1939). It is not possible to identify either of them with any previously described species, especially as they produce fluorescent pigment in a spasmodic and unpredictable manner only. They are, therefore, referred to as *Pseudomonas* sp., strain N 8, and *Pseudomonas* sp., strain 309.

Pseudomonas sp., strain N 8. Isolated from Harpenden soil by incubating a small quantity of soil in Giltay's medium, and plating on Giltay agar. Cultural characteristics: rods, about $1.0 \times 0.5 \mu$, motile with 1-2 polar flagella, non-spore forming, Gram-negative; agar streak: growth good, colourless, almost transparent, smooth, edge undulate, occasionally produces green fluorescent colour in agar, and also in liquid media. Liquefies gelatine rapidly. Produces acid but not gas on glucose, fructose and sucrose; lactose is not fermented. Reduces nitrates to nitrites and gaseous nitrogen. Slightly peptonizes litmus milk without change in acidity. Aerobic.

Pseudomonas sp., strain 309. Isolated from a small-scale septic tank in which milk washings were being treated in the laboratory. Cultural characteristics: rods, $1.0 \times 0.7 \mu$, motile with 1 polar flagellum, non-spore forming, Gram-negative; agar streak: growth good, colourless, translucent, faintly granular, edge undulate, occasionally produces green fluorescent colour in agar and in liquid media. Liquefies gelatine rapidly. Produces acid but not gas on glucose, fructose, galactose, mannitol and glycerol; lactose, sucrose and maltose are not fermented. Reduces nitrates to nitrites and gaseous nitrogen. Slightly peptonizes litmus milk without change in acidity. Can decompose fat. Aerobic.

METHODS

Media. The synthetic liquid media employed were made up in the following neutral basis solution (Klaeser, 1914):

KH_2PO_4	0.5 g.) in 1 l. distilled water.
K_2HPO_4	0.5 g.	
CaCl_2	0.1 g.	
NaCl	0.1 g.	
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.3 g.	
FeCl_3	Trace	

For alkaline media the mixture of phosphates was replaced by 1 g. K_2HPO_4 . The medium most commonly used also contained:

KNO_3	1.0 g. (139 mg. nitrogen) per litre.
Glucose	3.5 g. (1400 mg. carbon) per litre.

In some cases the nitrate was wholly or partly replaced by nitrite, to give about 140 mg. nitrogen/l., or increased in amount; but no other sources of nitrogen were used. Glucose was replaced in different experiments by a variety of organic compounds, all in sufficient quantity to give 1400 mg. carbon/l., and so a carbon/nitrogen ratio of 10. Organic acids were used as the sodium salt; and ethyl alcohol, when used, was added after steaming. The basis solution and nitrate were autoclaved, the glucose added and the pH adjusted to 7.0 or 8.0; the complete medium was measured out into the flasks to be used in the experiment, and steamed for 1 hr.

Inoculation and incubation

In two experiments the medium was contained in test tubes with Durham tubes, which were inoculated directly from young agar slope cultures. In all other experiments the medium was contained in conical flasks, and was inoculated as follows (Meiklejohn, 1937): a heavy suspension of the growth from a young agar slope was made in 10-25 ml. of sterile basis solution, and the number of cells counted. The volume of this suspension required to give an initial count of about 10 million bacteria/ml. was then pipetted into each flask. All flasks were incubated at room temperature and in the light, and were maintained at three levels of air supply, referred to as aerated, control and anaerobic conditions respectively. Control flasks were plugged with cotton-wool and were undisturbed except for sampling. They contained a fairly shallow layer of medium, not exceeding 2 cm. in depth. Aerated flasks were of the same size, and contained the same volume of medium, as the controls. The cotton-wool was replaced by a sterilized rubber stopper carrying inlet and outlet air tubes, and a small steady stream of air, filtered through cotton-wool, was bubbled through the medium. Anaerobic flasks were smaller and therefore contained a deeper layer of medium. They were stoppered with cotton-wool, and were incubated in a closed vacuum desiccator containing alkaline pyrogallol, under reduced pressure. Samples were taken from all flasks at the beginning and end of incubation, and usually during its course.

Examination of samples

Number of cells. The bacteria were counted throughout by the direct method with a Thoma haemocytometer. Duplicate counts were always made. This method of counting includes all bacterial cells present, whether viable or not. *pH determinations* were made colorimetrically with a Hellige comparator. *Nitrite* was detected by the Griess-Ilosvay reagent, and estimated by comparing a 1 ml. sample against a set of dilutions of a standard solution of sodium nitrite. If the nitrite test was negative, *nitrate remaining* in the medium was tested for by adding zinc dust to the nitrite sample and reagent, as suggested by ZoBell (1932).

The nitrogen estimation methods were primarily chosen for use with very small samples, as it was desired to alter the volume of medium as little as possible during the course of an experiment. All analyses were done in duplicate. *Total nitrogen* was determined, on 1 ml. samples, by a modified Kjeldahl method. If nitrate was present in the samples, it was first reduced with iron in acid solution, after the method of Pucher *et al.* (1930). The sample was then digested with concentrated sulphuric acid and a little potassium sulphate and copper sulphate; and the ammonia formed was estimated by Woolf's method (1928), in which the ammonia is expelled by aeration in presence of excess of alkali, caught in boric acid solution containing brom-cresol green, and titrated directly with sulphuric acid made up in the same boric acid-indicator solution. The strength of the acid used for titration was about $N/200$ (1 ml. = 0.0715 mg. N). *Non-protein nitrogen* was determined by the same method, after precipitation with basic lead acetate (Stiles *et al.* 1926). Blank determinations were made on the complete reagents used in each method. The blank values obtained were: *Kjeldahl*: 0.011 mg. N/ml. *Reduction* followed by *Kjeldahl*: 0.025 mg. N/ml. *Protein precipitation* and *Kjeldahl*: 0.015 mg. N/ml. These blanks have been subtracted from all the nitrogen values given in the tables and text-figures.

RESULTS

A. Effects of various organic compounds

Both species grew abundantly on the simple synthetic media used, with nitrate as the only source of nitrogen, if a suitable organic compound was supplied as a substrate. Several organic compounds were found to act as substrates for growth; but not all would act as hydrogen donors for the denitrification reaction. The only certain proof of denitrification is actual measurable loss of nitrogen; but as nitrogen gas is the end product, and nitrite an intermediate product, of denitrification, the appearance of gas in a culture, or the appearance and subsequent disappearance of nitrite, are indications that the reaction has taken place. The appearance of gas was regarded as an indication of denitrification in the following test-tube experiment; both species were grown on several different sugars, and on sodium lactate, under the following conditions: air supply *control*, pH 7.0, KNO_3 0.1 %, C/N ratio 10.

Table 1

Species	Days	Glucose		Sucrose		Galactose		Lactose		Lactate	
		NO ₂	Gas	NO ₂	Gas	NO ₂	Gas	NO ₂	Gas	NO ₂	Gas
309	3	0	+	6	0	60	0	6	0	60	+
	7	0	+	1	0	0	+	7	0	0	+
N 8	3	0	+	0	+	.	.	3	0	2	+
	7	0	+	0	+	.	.	15	0	0	+

NO₂ as mg. N/l.

Visible growth was seen in all the tubes, and from the appearance of gas it is evident that both species denitrify on glucose and lactate, N 8 on sucrose and 309 on galactose. It is also evident that neither strain will denitrify on sugars it does not ferment; 309 does not ferment sucrose, and only produces a little nitrite, and no gas, on sucrose. Neither strain ferments lactose, and does not denitrify on it.

Figs. 1 and 2 give the results of another set of experiments, in which species 309 was grown on six different organic compounds under these conditions: air supply *control*, pH 7.0, KNO_3 0.1 %, C/N ratio 10. In this case the formation and disappearance of nitrite was taken as an indication of denitrification; as no nitrate was left in the medium after the nitrite had disappeared, it is fairly safe to assume that denitrification really did take place.

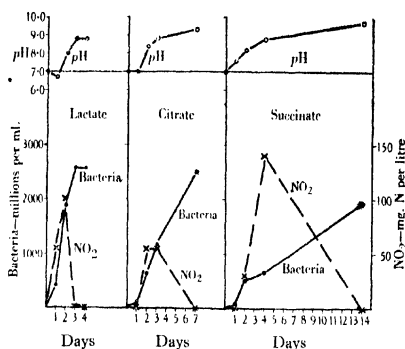


Fig. 1.

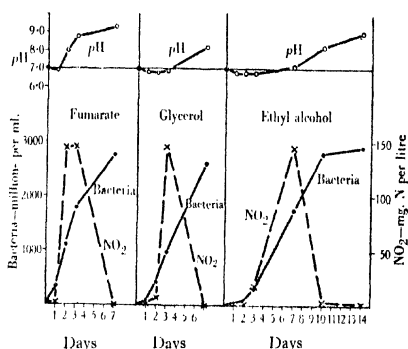


Fig. 2.

Fig. 1. Growth of species 309 and apparent denitrification on lactate, citrate and succinate.

Fig. 2. Growth of species 309 and apparent denitrification on fumarate, glycerol and ethyl alcohol.

The nitrite curves in Fig. 1 show that the denitrification reaction has taken place in all three cases. Species 309 can apparently use lactate, citrate, and succinate not only as substrates for growth, but also as hydrogen-donators in the reduction of nitrate. In each case the pH values show an alkaline drift, though the final pH was not the same. Species N 8 was also grown on lactate with almost identical results.

Three more compounds, sodium fumarate, glycerol and ethyl alcohol, can be used as hydrogen donors for the reduction of nitrate by species 309. On alcohol, growth was a little delayed, but high numbers of bacteria were finally produced on all three compounds; there was an alkaline drift in pH in the later stages after an initial slight acidity. Figs. 1 and 2 therefore include six cases in which nitrate was apparently completely reduced under aerobic conditions; the reduction was accompanied by good growth of the bacteria and an alkaline pH drift.

Fig. 3 gives three cases in which complete denitrification did not take place. Two of the experiments illustrated were performed under control air supply, and the third in anaerobic conditions; other conditions were: pH 7.0, KNO_3 0.1 %, C/N ratio 10.

The first case was a culture of 309 on glucose; when the experiment recorded in Table 1 was performed, 309 could denitrify in presence of glucose, but shortly afterwards it lost that power. When it was grown on glucose the medium became very acid, and nitrite accumulated and did not disappear. The total bacterial count was 800 millions/ml. at the end of the experiment, but it probably included very few viable cells, as attempts to sub-culture from this flask were unsuccessful. Another attempt was made to grow 309 on glucose, and in this case a pH value of 4.1 was reached in the culture after 4 days' incubation. The culture was then titrated back to pH 6.2 with weak alkali, but no further growth or reduction of the accumulated nitrite resulted. Glucose cultures of species N 8, on the other hand,

showed normal growth and complete reduction of the nitrate; a slight shift of *pH* to the acid side was always observed in the early stages; but no value more acid than 6.2 was ever observed. In the second case, a culture of 309 on malate, the cause of the failure of denitrification must be different, for the bacteria grew well, and a very alkaline *pH* value was attained. The third case of failure to denitrify is an anaerobic culture of species N 8 on ethyl alcohol; under the same conditions 309 grows and denitrifies normally; but N 8 hardly grows at all, and merely reduces all the nitrate to nitrite and no further. In all three cases nitrite accumulates in the medium; apparently the first stage of denitrification—the

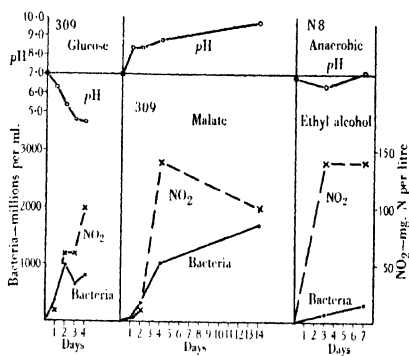


Fig. 3.

Fig. 3. Three cases of failure of denitrification.

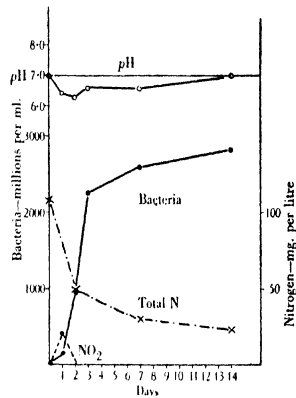


Fig. 4.

Fig. 4. Course of events in a glucose-nitrate culture of species N 8 under control air supply.

formation of nitrite—is less sensitive to external conditions than the subsequent stages. A case where even the first stage of reduction did not take place was observed in a triple experiment in which an attempt was made to grow species N 8 on sodium acetate (0.793 %, KNO_3 0.1 %, C/N ratio 10, *pH* 7.0). One week after inoculation the numbers of bacteria, from an initial count of 9 million/ml., had only risen to 10 million in the aerated, 12 million in the control and 31 million in the anaerobic culture; the only change in *pH* was from 7.0 to 6.9 in the anaerobic flask, and no nitrite at all was found, but much nitrate was still present.

Not only the kind but also the quantity of substrate influences the course of denitrification, as is shown in Table 2, which gives the results of an experiment on wide variations in the C/N ratio, performed under the following conditions: air-supply control, *pH* 7.0, C-source sodium lactate, N-source KNO_3 . The medium with C/N ratio 10 contained 0.1 % KNO_3 and 0.35 % lactic acid; these concentrations were varied to give the different ratios shown in Table 2.

Small quantities of nitrate are very rapidly reduced if the carbon source is present in sufficient quantity; but a deficiency in carbon source diminishes the growth of the bacteria, and at a C/N ratio of unity denitrification is not complete, and nitrite is left in the culture.

Table 2. 309 at different C/N ratios

Medium contains:

C mg./l.	N mg./l.	C/N	Days	NO ₂ mg. N/l.	Gas	Growth
1400	139	10	1	60	o	Good
			7	o	+	
700	139	5	1	96	o	Fair
			7	9	+	
140	139	1	1	48	o	Poor
			7	48	+	
1400	70	20	1	70	o	Good
			3	o	+	
1400	28	50	1	25	o	Good
			3	o	+	

B. *Effect of initial pH*

To find the limits of pH value outside which denitrification could not take place, species 309 was grown in test-tubes on media made up at various initial pH levels; the mixture of phosphates in the basis solution was altered to give solutions buffered at the desired level. The other conditions of the experiment were: air-supply *control*, KNO₃ 0.1 %, C-source sodium lactate, C/N 10.

Table 3. *Effect of initial pH on species 309*

Initial pH	Days	Final pH	Growth	NO ₂ mg. N/l.	Gas	Nitrate left
3.9	3	<4.0	Just visible	o	o	+
	7	<4.0	Just visible	o	o	+
4.8	3	7.0	Good	100	o	.
	7	7.4	Good	o	+	o
5.4	3	6.6	Good	100	o	.
	7	7.4	Good	9	+	o
7.0	3	8.2	Good	o	+	o
	7	8.4	Good	o	+	o
9.5	3	>9.0	Just visible	o	o	+
	28	>9.0	Poor	60	o	.

To judge by the appearance of gas, denitrification was complete in 3 days at initial pH 7.0. At initial pH 4.8 and 5.4 it was delayed, but at the end of 3 days the pH had shifted far enough to the alkaline side for denitrification to proceed normally. At the extreme pH values of 3.9 and 9.5 denitrification did not take place. In later experiments denitrification took place normally in media at initial pH 8.0 (cf. Karlsen, 1938).

C. *Effect of air supply*

The question of the existence of denitrification under aerobic conditions is of interest; examples have already been given in Figs. 1 and 2 of apparent denitrification under control (undisturbed aerobic) conditions, but as no total nitrogen estimations were performed, it is not certain that there was an actual loss of nitrogen in these experiments. A fresh series of experiments was set up to investigate the effect of air supply in more detail; in this series conditions other than air supply were kept as constant as possible. The same species—N 8—was used throughout, and the medium had the same composition: glucose 0.35 %, KNO₃ 0.1 %, C/N ratio 10, initial pH 7.0.

The results of a few examples from this series of experiments are given below. Determinations of total nitrogen were performed, so that any loss of nitrogen could be measured.

It was therefore possible to show with certainty whether denitrification did or did not take place. In Fig. 4 are given the data from a glucose-nitrate culture of species N 8 incubated under control conditions of air supply.

The curve of total nitrogen shows that there was a real loss of nitrogen, amounting to more than three-quarters of that originally present, from this culture grown as a shallow layer of liquid in an undisturbed aerobic flask. The rate of nitrogen loss was greatest in the first few days of incubation. The loss of nitrogen was accompanied by those changes in the culture that have already been indicated as signs of denitrification: (1) the bacteria grew well, (2) there was an early shift of *pH* to the acid side (characteristic of glucose cultures) followed by an alkaline drift, (3) nitrite was formed on the first day and then disappeared, (4) there was no nitrate left at the end of incubation, (5) on the second and third days of incubation, the culture had a "soda-water" appearance, caused by small bubbles of gas. Fig. 5 illustrates an experiment in which a control culture was compared with an aerated culture (through which air was bubbled).

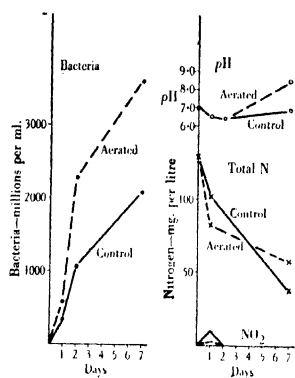


Fig. 5.

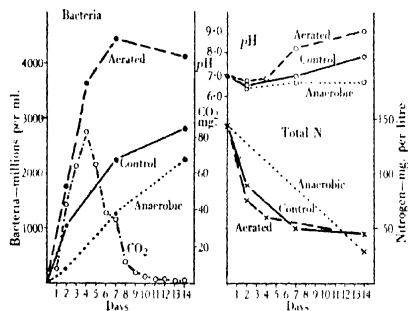


Fig. 6.

Fig. 5. Comparison of an aerated with a parallel control culture of species N 8.

Fig. 6. Comparison of three parallel glucose-nitrate cultures of species N 8: aerated, control and anaerobic.

In the first place it is evident that there was a considerable loss of nitrogen from both cultures, but slightly more nitrogen was left in the aerated culture at the end of the experiment than in the control. The numbers of bacteria were much higher in the aerated culture than in the control, and the final *pH* was more alkaline. These two effects of increased air-supply are also shown in Fig. 6, which gives the results of a three-fold experiment on aerated, control and anaerobic cultures.

More than two-thirds of the total nitrogen originally present was lost from all three cultures; least nitrogen was left in the anaerobic culture at the end of incubation. The numbers of bacteria were highest in the aerated culture, and lowest in the anaerobic, throughout the experiment; in the first few days of incubation there was an easily visible difference in turbidity between the aerated and anaerobic flasks. As in the last experiment, the growth of the bacteria appears to be stimulated by increased air-supply. The final *pH* value was progressively more alkaline as the air-supply was increased; subsequent experiments suggest that the difference in *pH* was probably due to the difference in amount of glucose used aerobically and anaerobically at a C/N ratio of 10. The carbon dioxide output of the aerated

culture was estimated by the Pettenkoffer method. In the 14 days of the experiment 371 mg. CO₂ were given off. A curve showing the rate of output of CO₂/24 hr. is included in Fig. 6; there is a sharp peak in the curve on the 4th day, towards the end of the logarithmic period of growth. Nitrogen was also lost most rapidly from the aerated culture in the earlier days of incubation. From the CO₂ curve it is evident that metabolic activity in the culture practically ceased after 10 days; it may therefore be assumed that the nitrogen estimation on the 14th day is a real measurement of the nitrogen left when the reactions accompanying growth were over.

Two examples have now been given where the numbers of bacteria were apparently increased by increased aeration; and this difference in numbers appeared throughout this series of experiments. To examine the reality of the difference, all the figures available were collected and compared. The difference between the bacterial counts taken on the same day from parallel cultures was set down; and the mean value of all the differences between control and anaerobic, aerated and control, and aerated and anaerobic parallels, was calculated, and an estimate made of the standard error of each of the three means.

Table 4. *Comparison of bacterial numbers at three aeration levels*
(all numbers in millions/ml.)

Exp.	Age in days	Anaerobic cultures	Difference control-anaerobic	Control cultures	Difference aerated-control	Aerated cultures	Difference aerated-anaerobic
7	1	—	—	336	+ 242	578	—
	2	—	—	1058	+ 1230	2288	—
	7	—	—	2072	+ 1528	3600	—
8	1	—	—	242	+ 90	332	—
	2	—	—	1014	+ 508	1522	—
	7	—	—	2925	+ 1175	4100	—
9	2	—	—	648	+ 592	1240	—
	3	576	+ 772	1348	+ 2342	3700	+ 3124
	16	1410	+ 2140	3550	+ 850	4400	+ 2990
12	2	248	+ 800	1048	+ 700	1748	+ 1500
	7	1260	+ 980	2240	+ 2210	4450	+ 3190
	14	2240	+ 585	2825	+ 1300	4125	+ 1885
23 A	14	1490	+ 1070	2560	—	—	—
	14	1520	+ 3380	4900	—	—	—
Number of cases			7		12		5
Mean value of difference			1400		1064		2538
Standard error of mean			± 383.3		± 205.8		± 351.9
<i>t</i> (Fisher, 1936)			3.65		5.17		7.21

The values of *t* in each column are high enough to be significant for that number of cases. That is to say, each mean value is sufficiently large, when compared with its own standard error, to leave no doubt of its real existence. It can therefore be regarded as certain that, in this series of experiments, the numbers of bacteria are higher in control than in anaerobic cultures, and higher in aerated cultures than in either of the two other classes.

As well as the difference in bacterial numbers, a difference in the amount of nitrogen left in the cultures at the end of the experiment appears in Figs. 5 and 6; and in several other experiments it was observed that most nitrogen was left in aerated, and least in anaerobic, cultures. At the end of these experiments, a determination of the nitrogen left after precipitation with basic lead acetate—"non-protein nitrogen"—was made. By subtracting this figure, usually small, from that for total nitrogen, an estimate can be made of "protein"

nitrogen, which is presumably the nitrogen locked up in the cells of the bacteria. The following mean values for "protein" nitrogen were obtained from cultures with a C/N ratio of 10: aerated cultures (4 cases) 43 mg. N/l.: control cultures (7 cases) 31 mg. N/l.: anaerobic cultures (3 cases) 22 mg. N/l. There are not many figures available, and too high a value should not be placed on the accuracy of the "protein" nitrogen estimations, as they represent the difference of two determinations on small samples. All the available figures for "protein" nitrogen, and the numbers of bacteria present at the same time, are given in Table 5.

Table 5. *Bacterial numbers and precipitable nitrogen*

Experiment	Flask	Number of bacteria 100 millions/ml.	"Protein" nitrogen, mg./l.
5	Control	28.25	20
7	Control	20.72	30
	Aerated	36.00	57
8	Control	29.25	27
	Aerated	41.00	39
9	Anaerobic	14.10	14
	Control	35.50	29
	Aerated	44.00	53
12	Anaerobic	22.40	18
	Control	28.25	25
	Aerated	41.25	22
25	Control	25.60	42
10	Anaerobic	21.10	31
	Control	25.55	53
	Aerated	30.40	66
26	Control	52.00	45
24 A	Anaerobic	15.55	34
B	Anaerobic	24.95	39
C	Anaerobic	39.70	53
D	Anaerobic	55.40	83

On the whole, low values for precipitable nitrogen correspond to low bacterial numbers, and high values to high numbers. To test whether a direct mathematical relation between the two quantities does in fact exist, the regression of precipitable nitrogen on bacterial numbers was calculated.

If x = numbers of bacteria in 100 millions/ml. and y = precipitable nitrogen in mg./l., then

Mean value of y = 39, if x	= 31.549.
Regression coefficient of y on x	= +0.9131.
Estimated standard error of regression coefficient	= 0.2984.
t	= 3.0601.
Number of cases	= 20.

From a table of t (Fisher, 1936) it can be found that the regression coefficient differs significantly from zero; this shows that a direct linear relation between x and y does in fact exist.

This relation can be expressed by the equation

$$y = 10.183 + 0.913x.$$

Fig. 7 shows the calculated regression line, with the observed points, which are evenly distributed about it. It may reasonably be concluded that the greater amount of nitrogen remaining in aerated cultures after denitrification, and hence the smaller loss of nitrogen under aerobic conditions, is directly connected with the greater growth of the bacteria. The

denitrifying reaction was not always completed, as in several cases measurable amounts of "non-protein" nitrogen were left at the end of an experiment; but a state was approached in which, in the presence of an adequate amount of an available organic compound, only the nitrogen required for bacterial cell-substance was retained in the culture, and all the rest was lost as gas. This seems to be a much simpler and more satisfactory explanation of the smaller loss of nitrogen under aerobic conditions than the "diminished denitrifying power" postulated by earlier authors (cf. Seiser & Walz, 1925).

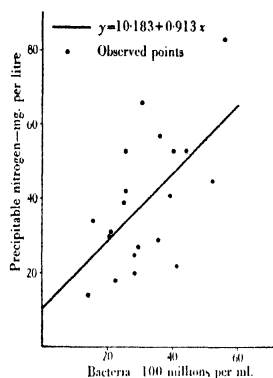


Fig. 7. Regression of precipitable nitrogen on bacterial numbers.

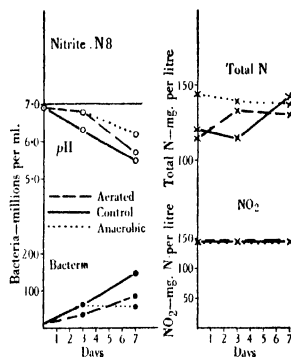


Fig. 8. Effect of nitrite on species N 8 in a neutral medium.

D. The reduction of nitrite

The reduction of nitrate by bacteria is divided into at least two stages: (1) the reduction of nitrate to nitrite, (2) the further reduction of nitrite. Nitrite is toxic to various organisms; yet in the normal course of denitrification the bacteria seem to be able to deal with fairly high concentrations of nitrite. It is, therefore, interesting to examine the effect on the bacteria if nitrate in the medium is replaced by nitrite. In the first experiment, all the nitrate in the medium was replaced by an equivalent amount of nitrite (140 mg. N/l.): the conditions of the experiment were: species N 8, air supply *all three levels*, pH 6.9, glucose 0.35 %, NaNO_2 0.069 %, C/N ratio 10 (Fig. 8). In all three cultures, the nitrite reaction was as strong at the end of the experiment as at the beginning; in a neutral medium, nitrite added at the beginning is not used by the bacteria. There was very little growth, the highest count being 146 million cells/ml. in the control culture. There was no loss of total nitrogen (the apparent loss in the anaerobic culture is due to irregularities in the analysis, which was affected by nitrite). The final pH values were slightly acid in all three cases.

In order to find out whether the bacteria did not reduce nitrite because they could not use it for growth, or if it was actually poisonous to them, a second experiment was set up in which nitrate and nitrite were both present in the medium. The total nitrogen of the media was the same (139 mg. N/l.), but varying proportions of the nitrate were replaced by nitrite. Other conditions were: species N 8, air supply *control*, pH 6.9, glucose 0.35 %, C/N ratio 10 (see Table 6).

Table 6. *Effect of increasing doses of nitrite in neutral media*

Mg. N/l. of medium		Days	Bacteria millions/ml.	pH	NO ₂ mg. N/l.	Total N mg./l.
As NO ₃	As NO ₂					
125	14	0	11	6.9	14	(139)
		14	3000	7.1	0	18
104	35	0	11	6.9	35	(139)
		14	1388	4.9	28	88
90	49	0	11	6.9	49	(139)
		14	848	4.9	<49	103
69	70	0	11	6.9	70	(139)
		14	332	4.7	70	111

These results show that nitrite was not reduced by species N 8 because it is toxic, and not merely because it cannot be used for growth. In all these media there is enough nitrate present to supply nitrogen for growth, but growth is diminished in all but the first medium. The toxic effects of nitrite increase with its concentration; species N 8 appears to tolerate nitrite equivalent to 14 mg. nitrogen/l., as at this concentration growth and denitrification are normal; but both processes are progressively affected by higher concentrations of nitrite in a neutral medium.

In an alkaline medium, however, the state of affairs is quite different. Karlsen (1938) pointed out that the optimum pH for the reduction of nitrite by *Ps. aeruginosa* is 8.1-8.6 for the highest concentration used; her strain also would not decompose nitrite in media more acid than pH 7.2. In consequence of Karlsen's results, the action of nitrite on species N 8 and 309 in alkaline media was investigated. Table 7 gives the results of a double experiment in media with initial pH 6.9 and 8.0: other conditions were: species 309, air supply *anaerobic*, glycerol 0.358 %, C/N ratio 10.

Table 7. *Effect of increasing doses of nitrite on 309*

Mg. N/l. of medium		Days	Neutral			Alkaline		
			Bacteria millions/ml.	pH	NO ₂ mg. N/l.	Bacteria millions/ml.	pH	NO ₂ mg. N/l.
As NO ₃	As NO ₂							
139	0	0	10	6.9	0	10	8.0	0
		7	2850	7.3	0	2695	7.2	0
104	35	0	10	6.9	35	10	8.0	35
		7	2100	7.4	0	2320	7.3	0
69	70	0	10	6.9	70	10	8.0	70
		7	280	6.7	70	1820	7.3	0
0	140	0	10	6.9	140	10	8.0	140
		7	26	6.6	140	1760	8.0	0

The total nitrogen estimations were seriously affected by the presence of nitrite, and are not included in Table 7; but the figures in the nitrite column show whether it was reduced or not. In the neutral medium, the higher concentrations of nitrite are progressively toxic to species 309, and are not reduced. But in the alkaline medium, species 309 will grow normally and denitrify on all the concentrations of nitrite given, even where nitrite is the only source of nitrogen in the medium. At the highest concentration of nitrite the number of bacteria is slightly diminished, but this is the only indication of any toxic action at all.

Species N 8 also is not poisoned by nitrite in alkaline solution; the results of an experiment in which nitrate alone was compared against nitrite alone are given in Table 8: all the media contained about 140 mg. nitrogen/l. and had a C/N ratio of 10.

Table 8. *Nitrite in alkaline solution—species N 8*

	Medium			Days	Bacteria millions/ml.	pH	NO ₂ mg. N/l.	Total N mg. N/l.
	Reaction	Nitrogen source	Carbon source					
Air-supply Control	Alkaline	NO ₃	Glycerol	0	11	8.0	0	114
				7	1700	8.4	0	32
Control	Alkaline	NO ₂	Glycerol	0	11	8.0	140	119
				7	3020	8.4	0	34
Control	Alkaline	NO ₂	Glucose	0	11	8.0	140	109
				7	424	5.5	c. 100	74
Control	Neutral	NO ₂	Glycerol	0	11	6.9	140	123
				7	48	6.9	140	119
Anaerobic	Alkaline	NO ₃	Glycerol	0	12	8.0	0	151
				14	2415	8.4	0	90
Anaerobic	Alkaline	NO ₂	Glycerol	0	12	8.0	140	(Lost)
				14	1640	8.4	0	65

The results included in Table 8 can be summed up as follows: (1) in a neutral medium nitrite is poisonous to species N 8 in presence of glycerol. (2) N 8 will grow normally and reduce nitrate in an alkaline glycerol medium under both conditions of air supply. (3) The toxic action of nitrite is cancelled in alkaline solution in presence of glycerol. (4) If glucose is used as the substrate, nitrite still appears to be poisonous in an initially alkaline medium; the cause of this is probably the acid formed in the breakdown of glucose.

E. Utilization of the carbon source

All the preceding experiments have been mainly concerned with the nitrogen content of the cultures; it has been briefly indicated that nitrate reduction is affected by variations in the C/N ratio, but the effect on the carbon-source itself has not been considered. Glucose was used as substrate in a series of experiments at different C/N ratios, and its presence or absence determined, at the end of incubation, by Fehling's or Cole's test. The results obtained show an interesting difference between aerobic and anaerobic cultures of species N 8.

In the first experiment, the C/N ratio of the medium was 10 (glucose 0.35 %, KNO₃ 0.1 %, pH 7.1). Two control cultures of species N 8 were compared against two anaerobic cultures.

Table 9. *Glucose left at C/N ratio 10*

Air-supply	Days	Bacteria millions/ml.	pH	NO ₂ mg. N/l.	Total N mg. N/l.	Glucose at end of experiment
Control A	0	7	7.1	0	142	Absent
	14	2560	8.1	0	58	
Control B	0	7	7.1	0	142	Absent
	14	4900	7.9	0	47	
Anaerobic A	0	7	7.1	0	142	Present
	14	1490	8.5	0	75	
Anaerobic B	0	7	7.1	0	142	Present
	14	1520	9.0	0	43	

In all four cultures denitrification proceeded normally; and in the aerobic cultures all the glucose was used. But in the anaerobic cultures not all the glucose was used, and the numbers of bacteria were smaller. It seems, therefore, that at a C/N ratio of 10, enough

nitrate is present in the aerobic cultures for full growth and decomposition of the glucose; but in the anaerobic cultures both processes are limited by the amount of nitrate available.

A second experiment at C/N ratio 5, with twice the amount of nitrate, gave similar results (glucose 0.35 %, KNO_3 0.2 %, pH 6.8, species N 8).

Table 10. *Glucose left at C/N ratio 5*

Air-supply	Days	Bacteria millions/ml.	pH	NO_2 mg. N/l.	Total N mg. N/l.	Non- protein mg. N/l.	"Protein" mg. N/l.	Glucose at end of experiment
Aerated	0	10	6.8	0	235	—	—	—
	7	3040	8.6	0	66	0	66	
Control	0	10	6.8	0	237	—	—	
	7	2470	8.2	0	58	13	45	Absent
	14	2555	8.5	0	53	0	53	
Anaerobic	0	10	6.8	0	222	—	—	Present
	7	1800	8.2	0	33	9	24	
	14	2110	8.2	0	31	0	31	

Denitrification is complete in all three cultures, as is shown by the disappearance of "non-protein" nitrogen, but again at this C/N ratio there is not sufficient nitrate present in the anaerobic culture for the glucose to be completely used. The difference in numbers of bacteria is not quite so great as at C/N ratio 10. If the C/N ratio is narrowed still more by increasing the amount of nitrate with the same amount of glucose, no very striking changes are produced in cultures of N 8 under aerobic (control) conditions. An experiment on these lines showed abundant growth, alkaline drift in pH, loss of total nitrogen, and complete utilization of the glucose, at nitrate concentrations of 0.4 and 0.8 %; at the latter concentration (1109 mg. N/l., C/N = 1.25) there was a small amount of nitrite left in the medium after 15 days. The number of bacteria present at this concentration of nitrate was slightly smaller than the number in the parallel culture with 0.1 % nitrate. Under anaerobic conditions, on the other hand, the narrowing of the C/N ratio produces striking changes. The results of such an experiment with strain N 8 are given in Table 11.

Table 11. *Effect of C/N ratio in anaerobic cultures*

Conc. KNO_3 %	C/N ratio	Days	Bacteria millions/ml.	pH	NO_2 mg. N/l.	Total N mg. N/l.	Non- protein mg. N/l.	"Protein" mg. N/l.	Glucose at end of experiment
0.1	10	0	11	6.8	0	122	—	—	Present
		15	1555	8.6	0	60	26	34	
0.2	5	0	11	6.8	0	(278)	—	—	Present
		15	2495	8.8	0	72	33	39	
0.4	2.5	0	11	6.8	0	(554)	—	—	Absent
		15	3970	9.0	0	81	28	53	
0.8	1.25	0	11	6.8	0	(1109)	—	—	Absent
		15	5540	8.8	60	259	176	83	

The numbers of bacteria increase progressively as the nitrate concentration increases, from a value characteristic of those previously observed in anaerobic cultures at C/N 10, to a value as large as any observed in aerated cultures. It therefore appears probable that the differences in bacterial numbers between aerobic and anaerobic cultures at C/N 10 are caused by the limiting effect of the amount of nitrate available under anaerobic conditions. The amount of precipitable nitrogen increases with the nitrate concentration, roughly in

proportion with the bacterial numbers. At C/N ratios 10 and 5 there is not enough nitrate present for complete utilization of the glucose, and some is left at the end of incubation. At C/N ratio 2.5 the supply of nitrate is sufficient for the glucose to be completely used; and at C/N 1.25 there is a little too much nitrate, as is shown by the presence of nitrite at the end of the experiment, and by the large figure obtained for non-protein nitrogen, in contrast to the small and approximately constant quantity recorded in the other three cultures. Strain N 8, therefore, when grown in anaerobic conditions, requires more nitrate, relative to the amount of organic matter supplied, than it does in the presence of air.

DISCUSSION

Species N 8 and 309 have simple requirements for growth; both species can grow on a synthetic medium with nitrate as the only source of nitrogen, and the limiting pH values for species 309—3.9 and 9.5—are wide apart. A variety of organic compounds could be used as substrates for growth, but not all of them acted as hydrogen-donators for denitrification; failure of denitrification usually involved the later stages of the reaction only; the nitrate was reduced to nitrite, but no further.

The results of the experiments on aeration show that nitrogen is lost from cultures at all three levels of air supply. This loss is accompanied by the same changes in the culture that were recorded as signs of apparent denitrification in the first experiments; they include an alkaline drift in pH (preceded by slight acid formation in glucose cultures). As Weissenberg (1902) pointed out, true denitrification is always accompanied by an increase in alkalinity of the medium; and the pH values observed exclude the possibility of so-called "indirect" denitrification, i.e. the formation of nitrogen in the reaction between nitrite and ammonia or amines, since this reaction only takes place in acid solution. The cultures in several experiments were tested for ammonia with Nessler's reagent, but none was found. Nitrogen was always lost most rapidly in the first few days of the incubation period, and the reduction was complete after about 10 days' incubation at room temperature.

The amount of nitrogen remaining after the reduction of the nitrate was highest in aerated, and lowest in anaerobic, cultures; and most of it was "protein" nitrogen. Von Caron (1912) recorded that more protein was formed in aerated liquid cultures of three denitrifiers than in anaerobic cultures, and Seiser & Walz (1925) compared anaerobic and undisturbed aerobic cultures of *Ps. putida*, and found that consistently more nitrogen was retained in aerobic cultures. They associated this with increased bacterial growth; but they made no counts of cell numbers, being content with eye estimates of turbidity. In the present case, as counts showed that the numbers of bacteria increased with increased aeration, and as "protein" nitrogen was found to be directly proportional to bacterial numbers, the greater amount of nitrogen retained in aerated cultures can be attributed to greater growth of the bacteria. Less nitrogen is lost from aerated than from anaerobic cultures, not because the bacteria have a greater "denitrifying power" under anaerobic conditions, but because the larger numbers of bacteria in aerated cultures lock up more nitrogen in their cell proteins.

Karlsen (1938) showed that nitrite was toxic to *Ps. aeruginosa* in neutral or faintly acid solution, but was harmless and freely reduced to nitrogen in alkaline solution (optimum pH 8.1–8.6). Strains 309 and N 8 are also poisoned by nitrite at pH 6.9, even in the presence of nitrate, but freely reduce it at pH 8.0. The poisonous effect of nitrite at pH 6.9 is pro-

portional to the concentration; this fact, as Karlsen pointed out, invalidates the explanation given by Elema (1932) for the toxicity of nitrite. He attributed it to the high oxidation-reduction potential produced by the presence of nitrite in acid solutions. But, as Elema himself shows, the potential level in denitrifying cultures is dependent on the presence, and not on the concentration, of nitrite. It is possible, as Karlsen suggests, that free undissociated nitrous acid is considerably more poisonous than the nitrite ion.

The changes produced in anaerobic cultures of species N 8 by varying the C/N ratio make it evident that, when grown anaerobically, this species requires more nitrate, relative to the amount of energy source, than it does under aerobic conditions. This is explicable on the hypothesis that nitrate is used as a source of oxygen as well as a source of nitrogen by the organism growing anaerobically. In other words, anaerobic denitrification can be explained by the "oxygen need" theory. But this theory will not account for the fact of denitrification under aerobic conditions. When air is bubbled through the culture, the bacteria will still completely reduce all the nitrate present, and retain only the nitrogen used to build up their cell proteins. The enzyme system concerned in denitrification does not appear to be the same as the *nitratase* of Quastel *et al.* (1925), by means of which *Bact. coli* reduces nitrate to nitrite, and is enabled to grow anaerobically in presence of nitrate and a hydrogen-donor. For, as Stickland (1931) showed, the *nitratase* system of *Bact. coli* is inhibited by oxygen; a gas mixture containing 20.9 % oxygen (\equiv air) causes a 94 % inhibition, and the enzyme is perceptibly inhibited by very small amounts of oxygen (down to 0.36 %). It may, therefore, be said that aerobic denitrification undoubtedly takes place, but cannot be satisfactorily explained on any existing theory.

SUMMARY

1. Two species of *Pseudomonas* are described, which reduce nitrate to nitrite and nitrogen gas in simple synthetic media.
2. An adequate supply of a suitable organic compound is necessary for denitrification.
3. Both species will denitrify in aerated, and in undisturbed aerobic cultures, as well as under anaerobic conditions.
4. At C/N ratio 10, the bacteria grow to higher numbers in aerobic than in anaerobic cultures. The amount of precipitable nitrogen retained in a culture is directly proportional to the bacterial numbers, and therefore the smaller loss of nitrogen from aerobic compared with anaerobic cultures is a consequence of the greater growth of the bacteria.
5. At pH 6.9 nitrite has a poisonous effect, proportional to its concentration, on both species; but at pH 8.0 it is harmless and freely reduced.
6. Species N 8 requires more nitrate, relative to the amount of organic matter present, under anaerobic than under aerobic conditions.

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The structure of 'ineffective' nodules and its influence on nitrogen fixation

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[Plates 13, 14]

1. The anatomy and cytology of nodules produced on clover, peas and soy beans by 'effective' and 'ineffective' strains of *Rhizobia* were investigated, with especial reference to the changes in volume of the active infected tissue during the life of the nodule.

2. In clover the mean volume of this active bacterial tissue is about three times as great in 'effective' as in 'ineffective' nodules. This is due to an early arrest of growth in nodules produced by ineffective strains.

3. In all nodules the active bacterial tissue eventually disintegrates, but in effective clover nodules it remains without disintegration for about six times as long as in ineffective nodules.

4. In an experiment to test the nitrogen fixation by clover inoculated with an effective and an ineffective strain, the difference between the strains in the amounts of nitrogen fixed could be accounted for by the differences in volume and in duration of the active bacterial tissue.

5. In peas, nodules produced by an effective strain were nearly twice the

length of those produced by an ineffective strain, and their bacterial tissue remained without disintegration for about twice as long.

6. In soy beans the mean volume of bacterial tissue was 4.75 times as great in effective as in ineffective nodules and the percentage of that volume composed of infected cells was twice as great.

7. In ineffective soy bean nodules disintegration of the bacterial tissue began when the plant was 4 weeks old and was practically complete by the twelfth week, at which time no disintegration could be found in effective nodules.

8. The difference in amount of nitrogen fixed by soy bean plants bearing each type of nodule could be accounted for wholly by the factors mentioned above.

9. Thus in both clover and soy bean nodules the volume and duration of the active infected tissue are the main, if not the only, factors determining differences in nitrogen fixation amongst the strains tested.

A. INTRODUCTION

The fact that strains of nodule bacteria differed in their ability to benefit the host legume was realized as early as the nineties of last century. It was, however, the careful work of Stevens (1925) working with lucerne, and of Wright (1925*a, b*) with soy beans, that definitely showed the wide differences in nitrogen fixed in the same species of host legume when infected with different strains of bacteria. Their work has been amply confirmed and has been extended to other host plants by numerous workers such as Helz, Baldwin and Fred (1927) working with peas, Eckhard, Baldwin and Fred (1931) with lupins, and Baldwin and Fred (1929) with clover.

In some cases, especially amongst clover nodule bacteria, strains have been found that fix very small amounts of nitrogen and confer scarcely any benefit on their host. Such strains are here referred to as 'ineffective' although this term is relative and it is doubtful whether strains exist that fix no nitrogen at all.

Various authors have attempted to find some correlation between ineffectiveness and such other characters as serological behaviour and cultural features shown *in vitro*. Stevens (1925) and Wright (1925*a*) had some success in relating ineffectiveness to agglutination reactions, while Baldwin and Fred (1927) found that strains of lucerne nodule bacteria showed differences in respect to mannitol fermentation that agreed with the determinations of effectiveness in nitrogen fixation found by Stevens (1925). But, on the whole, little correlation has been found between effectiveness in the plant and behaviour of the organisms in laboratory culture.*

* Dr Hugh Nicol tested the strains of nodule bacteria that form the subject of this paper as regards their growth and the change of reaction produced in media containing a wide range of carbohydrates and higher alcohols. No differences were found that could be correlated with effectiveness towards the host plant.

It has, nevertheless, been natural to suppose that bacteria of ineffective strains fix less nitrogen in the host plant owing to some defect in the mechanism of nitrogen fixation possessed by them. This is not self-evident, however, since crude nitrogen determinations of host plants inoculated with different strains do not provide a measure of the nitrogen-fixing efficiency of the bacteria unless an estimate can be made of the quantities fixed by unit masses of bacteria in unit time. To obtain this estimate, corrections must be made for such factors as the number of nodules, the volume of active tissue containing bacteria within these nodules, and duration of its activity. Adequate data for making even approximate corrections of the last two factors have not hitherto been available. The need for such data has, however, long been apparent from the frequently recorded fact that both the numbers and mean size of nodules show large differences as between strains, those that are ineffective tending to produce more numerous but much smaller nodules.

Surprisingly few observations have been recorded in which the anatomy and cytology of nodules produced by effective and ineffective strains have been compared. Elizabeth McCoy (1929) studied the anatomy of nodules on *Phaseolus*. Earlier workers such as Frank (1890) and Schneider (1892) considered that nodules on *Phaseolus* as a class were not beneficial to the host plant. Benefits from inoculation have been recorded with *Phaseolus* by Wilson and Leland (1929) but it does seem that ineffective strains are particularly prevalent with this host plant. In any case, the nodules studied by McCoy were apparently on an ineffective type. She found that the formation of the nodule was due rather to the multiplication of infected cells than to that of uninfected cells which were later invaded by the bacteria, as is more usual in legume nodules. The former process, however, has been found by Milovidov (1926, 1928) to be typical of lupin nodules, an observation confirmed by us, and is hence more likely to be conditioned by the type of host plant than by the particular strain of the invading organism. McCoy also found her *Phaseolus* nodules to be unusual in the following characters. (1) There were relatively few infected cells in the central tissue. (2) The nodules contained an abundance of starch associated with a great development of mitochondria. (3) The bacteria were rod-shaped and did not change into swollen or branched 'bacteroids'.

Marie Löhnis (1930) studied the anatomy of pea and clover nodules, in each case produced by an effective and an ineffective strain. She found that the two types of pea nodules differed in the amount and distribution of starch, the ineffective nodules containing more starch, more widely distributed through the nodule. Her effective nodules also contained a peculiar type of bacterial cell not found in the ineffective nodules. In

clover nodules, however, she records no differences in anatomy, in amount and distribution of starch or in the shape of the bacteria as between effective and ineffective nodules. This similarity suggested that the unusual characters found in ineffective nodules on *Pisum* and *Phaseolus* are not necessarily associated with inefficiency.

It therefore seemed desirable to study the anatomy and cytology of nodules produced on several host plants by effective and ineffective strains, first, in order to determine what characters were common to ineffective but not found in effective strains, and, secondly, to obtain quantitative data from which it might be possible to form some estimate of the relative amount of nitrogen fixed by a unit mass of bacteria in unit time in nodules of each class. For without such an estimate it was impossible to determine how much of the ineffectiveness of a given strain was due to relative inability of the individual bacteria to carry out the process of nitrogen fixation, and how much to poor growth of the bacteria within the host tissues.

B. MATERIAL AND METHODS

The nodules on clover, peas and soy beans were chosen for this investigation, because these are widely different types of host plant from which strains of nodule bacteria have been isolated that differ greatly in effectiveness. The following bacterial strains were used:

Soy bean		} Supplied by the Wisconsin Agricultural Experiment Station.*
Effective strain	501	
Ineffective strain	507	
Pea		
Effective strain	310	
Ineffective strain B. 33		
Clover		
Effective strain	205	
Ineffective strain	202	

Effective strain A, supplied by the Agricultural Experiment Station, Stockholm.

Ineffective strain Coryn, obtained from hill pasture on Coryn Mountain, Aberystwyth.

* The authors are gratefully indebted to Professor I. R. Baldwin, Wisconsin Agricultural Experiment Station, and to Professor Christian Barthel, Landbrukshögskolan, Uppsala, for supplying the cultures referred to; also to the Staff of Aberystwyth University for the clover plants from which the Coryn strain was isolated.

Strains 205 and 202 are the same that formed the subject of Marie Löhnis' observations on clover nodules.

The original strains 205 and 202, obtained from Wisconsin, lost their power of producing nodules during the course of the work, although strain 205 has since recovered this power without loss of effectiveness. In the meantime, however, a second active culture of each strain was obtained from Wisconsin in 1937, and these are here distinguished by the numbers 2057 and 2027.

The anatomical descriptions given below are based on the study of nodules on clover grown in agar and on peas and soy beans grown in sand.

The clover plants used for this study were grown in agar media in test tubes $1\frac{1}{4}$ in. in diameter. Three methods were employed. The first was the usual one of growing the plants in agar blocks made by pouring about 30 ml. of the melted agar medium into each tube and allowing it to solidify with the tube kept upright. Seeds were sown near the glass so that a good proportion of the root system should remain visible. The second method was to pour 10 ml. of melted medium into each tube and to solidify it by rotating the tube under a stream of cold water, as is done in the 'roll-tube' method of plating bacteria. Seeds were sown at the upper edge of the film of medium. Such cultures have the advantage that the whole root system remains visible through the glass, so that the appearance and growth of the individual nodules can be followed. It was used in obtaining growth curves of nodules and to obtain nodules of known age for anatomical study. The method suffered from a tendency of the agar film to dry up. This drying could be delayed by adding a few ml. of sterile water to each tube, but it was later replaced by a third method in which 12 ml. of agar medium were added to each tube and allowed to solidify and form a slope, the seed being sown at the upper end.

For the first, or agar block method, the following medium was employed:

K_2HPO_4	0.5 g.	NaCl	0.1 g.	$FeCl_3$	0.01 g.
KH_2PO_4	0.5 g.	$Ca_3(PO_4)_2$	2.0 g.	Agar	10.0 g.
$MgSO_4 \cdot 7H_2O$	0.2 g.	$FePO_4$	0.5 g.	Tap water	1 l.

For the roll and slope cultures, the medium was modified as follows in order to stiffen the agar gel and to reduce the opaque phosphate precipitate:

K_2HPO_4	1.0 g.	$FeCl_3$	0.01 g.
$MgSO_4 \cdot 7H_2O$	0.2 g.	Agar	20.0 g.
$CaH_4(PO_4)_2 \cdot 2H_2O$	0.5 g.	Tap water	1 l.
NaCl	0.1 g.		

The tubes of agar were autoclaved for 20 min. at 15 lb. pressure. For inoculation, the tubes were cooled to 42° C and a loopful of the appropriate culture was added to the melted agar and mixed by gentle shaking. This method gives quicker and heavier infection of the plant than that of adding the bacteria after the agar has solidified.

The seeds were externally sterilized by shaking for 3 min. in absolute alcohol, for another 3 min. in 0.2 % HgCl_2 and washing in four changes of sterile water. Two to four seeds were planted in each tube with a flamed platinum loop. During growth, the tubes were supported in blocks of wood drilled with suitable holes of such a depth as to keep the root system shaded.

For nitrogen determinations, clover was grown in quart milk bottles each containing 800 g. of sand and 100 ml. of the following food solutions:

K_2SO_4	0.9 g.	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.5 g.	Boric acid	0.02 g.
K_2HPO_4	0.5 g.	NaCl	0.5 g.	MnSO_4	0.02 g.
$\text{CaH}_4(\text{PO}_4)_2 \cdot 2\text{H}_2\text{O}$	0.5 g.	FeCl_3	0.02 g.	Tap water	1 l.

The bottles of sand were autoclaved for 2 hr. and the food solution separately autoclaved for 15 min. at 15 lb. pressure. The inoculum was suspended in the food solution and the suspension added to the sand with a sterile pipette. Seven to ten externally sterilized seeds were sown in each bottle.

Peas and soy beans were grown in glazed earthenware pots, the former in small pots containing 3 kg., and the latter in large pots holding 12 kg. of sand. The pots of sand used for peas were rendered free from a contamination of nodule bacteria by blowing steam upwards through the sand for 14 min. via the hole at the base of the pot. In the case of soy beans the pots and sand were not sterilized, as these were found to be free from soy bean nodule bacteria.

The same food solution was used for peas and soy beans as for the bottle cultures of clover. 200 ml. of this solution was added to each of the small pots and 1000 ml. to each of the large pots. More food solution was added as required during the course of the experiments. The solution used in growing the peas was sterilized and watering was done with boiled water. The inocula were added to the first dose of food solution before adding this to the pots. Seeds of both peas and soy beans were externally sterilized and sown at a uniform depth. In all experiments with clover, peas and soy beans, uninoculated controls were grown and these remained free from nodules.

For anatomical study, nodules were fixed at intervals during the growth of the host plants and in the case of clover, nodules of known age were

taken. They were usually fixed in Allen's modification of Bouin's solution (Allen's P.F.A. 3) and occasionally in Flemming's medium solution (as given by McCoy 1929). They were brought through alcohols into chloroform, imbedded in paraffin wax and cut into sections 6–10 μ thick. Most of the sections were stained with Heidenhain's iron haematoxylin with or without a counter-stain of erythrosine or orange G.

For studying the distribution of starch, slides were treated with a mordant of 2 % aqueous solution of tannin for 12 hr., stained for 2 min. with 1 % aqueous gentian violet and differentiated in 95 % alcohol. Excellent results were obtained when this method was combined with the haematoxylin stain. The slides were then treated as above described after the haematoxylin had been differentiated with iron alum, and were finally counter-stained with orange G.

C. THE DEVELOPMENT AND STRUCTURE OF CLOVER NODULES PRODUCED BY DIFFERENT STRAINS OF BACTERIA

In agar cultures of clover, the nodules induced by the strains here studied, first appeared on plants about 8 days old, which had produced their first true leaves. Nodules visible to the naked eye appeared on the same day in which infected root hairs were first observed, indicating that the bacteria pass down the root hair and induce proliferation of the root cells within 24 hr. The early development of the nodule follows the course described by Thornton (1930*a*) for lucerne nodules. The first stage consists of a mass of proliferating cells (figure 1, plate 13) mostly in the cortex but penetrating into the pericycle. The central cells soon swell and most of them become infected with bacteria brought into them by the infection threads (figure 2, plate 13). At this stage also, vascular strands begin to be differentiated along the sides of the nodule. By the time the nodule is a week old the cytoplasm of the infected cells in the central region becomes filled with bacteria. This central tissue is referred to below as the 'bacterial tissue' (figure 3, plate 13).

At the distal end of the nodule a cap of cells remains meristematic. In all clover nodules studied, the bacteria in recently infected cells close behind this meristem cap are rod shaped, but in older parts of the bacterial tissue they change into branched or swollen forms, often referred to as 'bacteroids'. The shape of these bacteroids differs considerably in different strains but these differences are not correlated with the effectiveness of the strain. The bacteroids in nodules produced by strains 2057, A and 2027 are usually pear-shaped or branched, an observation already made for the two

American strains by Marie Löhnis (1930). In the Coryn strain the bacteria undergo a type of change never observed by the authors in any other nodules (figure 4). Swellings appear either at the end or in the centre of the young rod-shaped cells and increase until they absorb the whole of the rod, which is thus converted into a sphere. At first the staining material is distributed throughout the spherical cell but later this became collected to form a deeply staining granule. Final disintegration releases these granules from the cells.

The amount and distribution of starch varies very greatly in individual clover nodules but seems to bear no relation to the effectiveness of the strain.

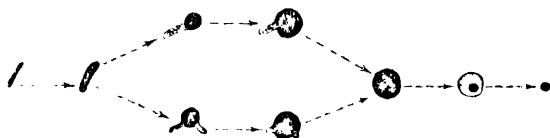


FIGURE 4. Formation of spherical forms by Coryn strain bacteria in the nodule.

The early course of nodule formation is common to all the strains studied, whether effective or ineffective. It results in the formation of an approximately spherical nodule about 0.5 mm. in diameter. From this stage, however, the course of events differs in effective and ineffective nodules.

In nodules produced by the effective strains 205, 2057 and A, the apical meristem continues its activity (figure 5, plate 13) and causes the nodules to grow in length so that, when 7 weeks old, they have a mean length of about 2 mm. (figure 6).^{*} The mean length of such nodules of all ages on plants ten weeks old is 1.45 mm. In nodules produced by the ineffective strains Coryn and 2027, the meristem cap ceases to function after about 7 days so that the nodules remain small and round, having a mean diameter of 0.8 mm. In these nodules there is therefore a very small volume of bacterial tissue (figure 3, plate 13). The volume of this tissue in clover nodules bears a fairly constant relationship to their overall length, as was also found to be the case with lucerne (Thornton and Nicol 1936). This relationship, found for nodules of strain 2057 and Coryn, is shown graphically in figure 7, which is based on measurements of nodules from plants of varying age grown in agar and on the assumption that the bacterial tissue is cylindrical in shape. The mean length of strain 2057 nodules on plants 10 weeks old corresponds to a bacterial tissue volume of 0.22 cu. mm.,

^{*} The data plotted in figure 7 are derived from the agar cultures described below in § D (p. 218). The volume of organized bacterial tissue plotted in figure 10 were also obtained from these cultures.

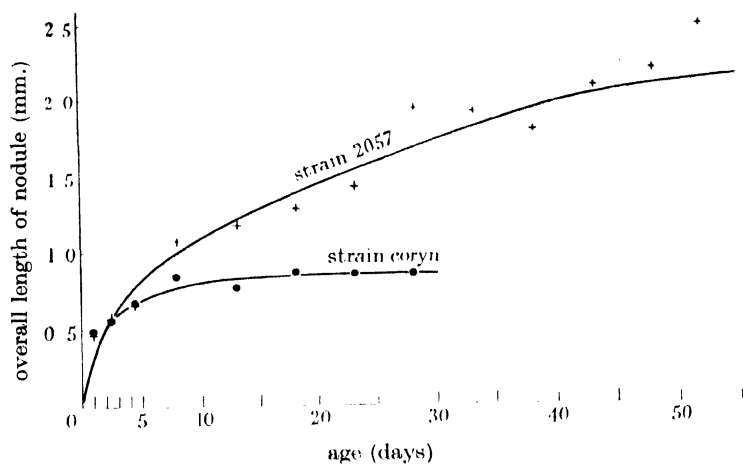


FIGURE 6. Growth in length of effective and ineffective clover nodules.

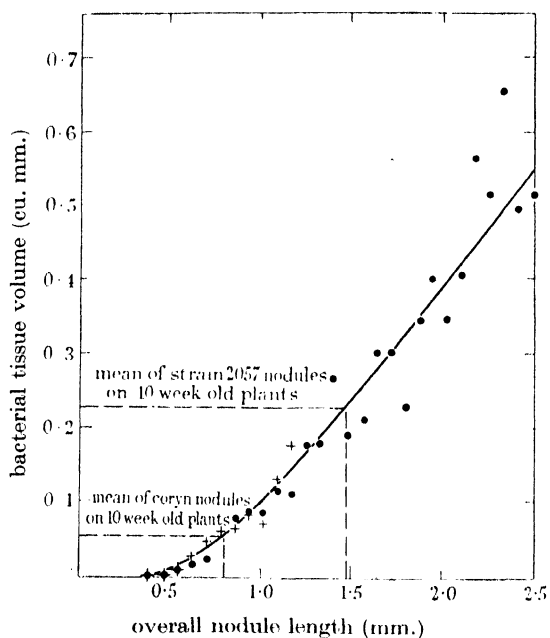
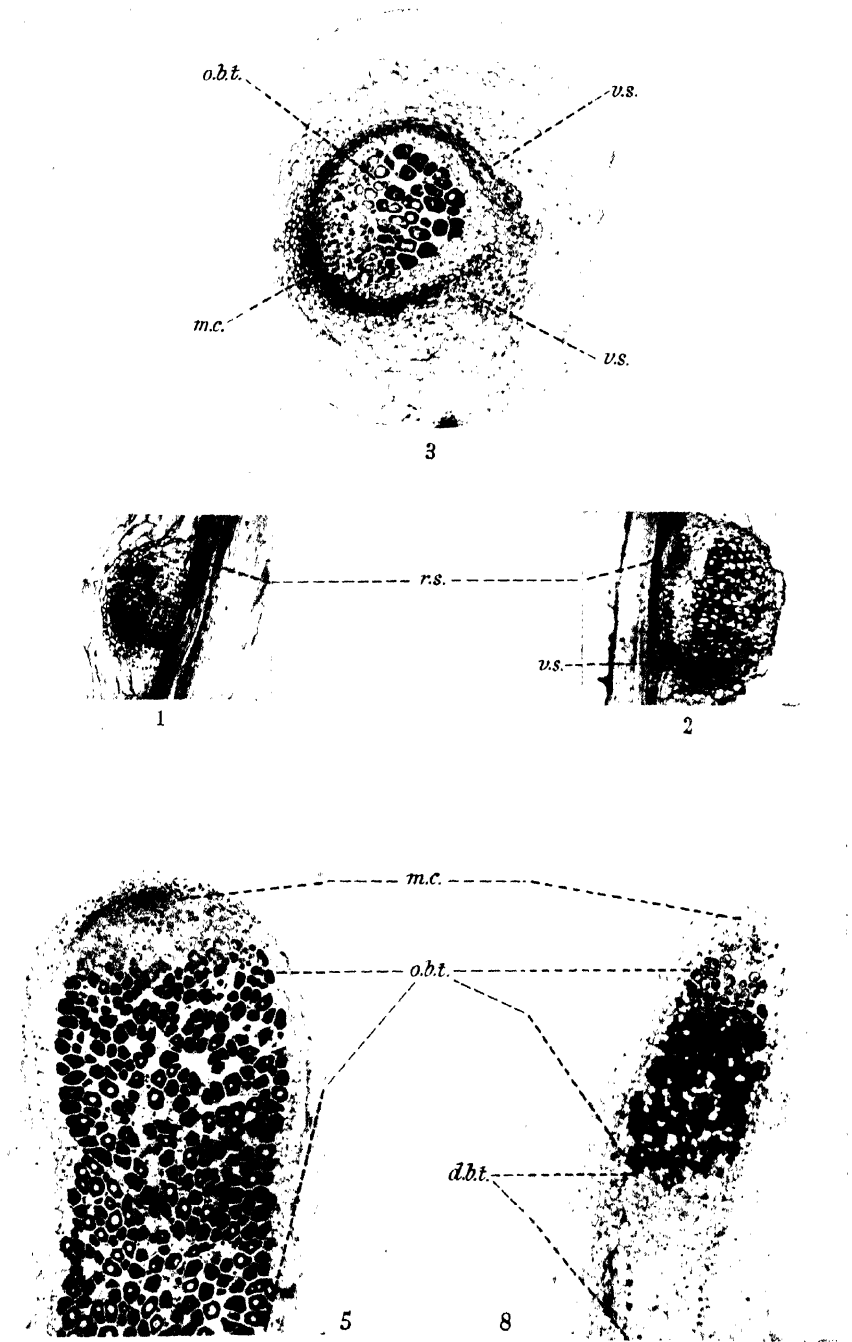
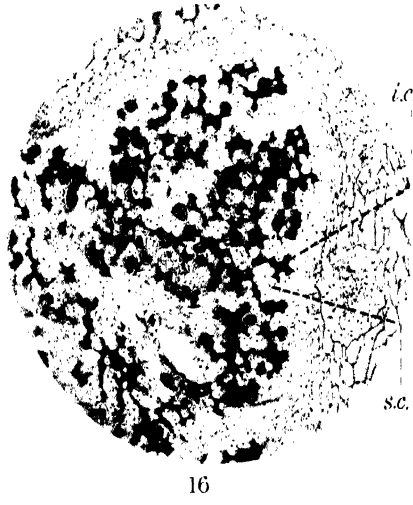
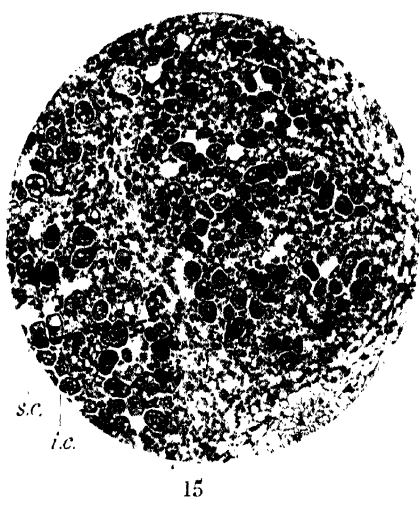
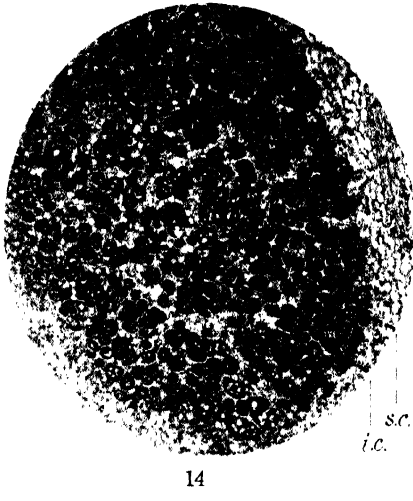
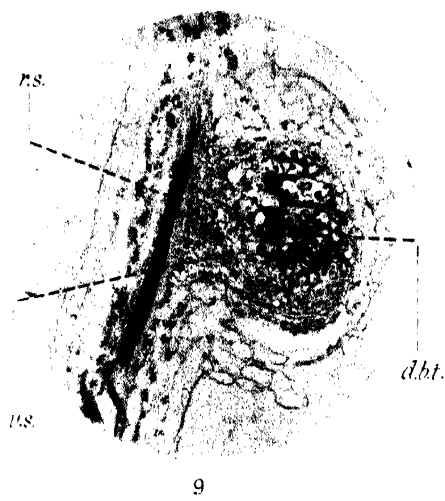


FIGURE 7. Relation of overall length to volume of bacterial tissue in clover nodules. Each point gives the mean volume of bacterial tissue in from 3 to 10 nodules of the same overall length. Dots refer to strain 2057, crosses to the Coryn strain nodules.





whereas that of Coryn nodules corresponds to a bacterial tissue volume of only 0.05 cu. mm.

In clover nodules of all the strains studied, the bacterial tissue eventually disintegrates owing to parasitic attack on the tissues by the bacteria, as has been described in the nodules on clover and lucerne by Thornton (1930*b*). But one of the most striking differences between effective and ineffective nodules is the time at which this disintegration takes place. In nodules produced by the effective strains, the bacterial tissue begins to disintegrate at the base, when the nodule is about a month old (figure 8, plate 13).

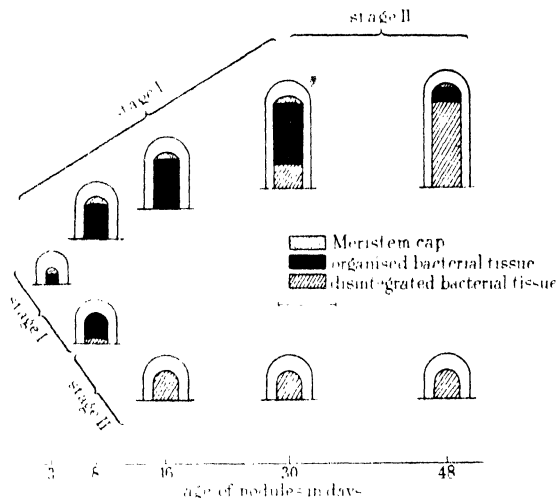


FIGURE 10. Growth and decay of the bacterial tissue in clover nodules.

After about eight weeks the centre of the nodule usually shows complete disintegration and is filled with collapsed and necrotic cells. In ineffective nodules of strains 2027 and Coryn, disintegration begins when the nodule is only about 7 days old, that is, almost as soon as the bacterial tissue is developed, and is complete by about the 15th day (figure 9, plate 14).

Measurements of the volume of total bacterial tissue such as are plotted in figure 7, therefore give an incomplete picture of the differences between effective and ineffective nodules, for not only does the volume attained by the bacterial tissue differ in the two types of nodule, but also the duration of its active life prior to disintegration. It is the differences in volume of organized, or undisintegrated bacterial tissue throughout the life of the nodules that are of importance. These differences are illustrated diagrammatically in figure 10. The history of the bacterial tissue in a nodule can be divided into two stages. During stage I, which lasts from its first appearance

until disintegration commences, the volume of the organized bacterial tissue is increasing. During stage II, the progress of disintegration from the base upwards causes a decrease in the volume of the organized bacterial tissue, which is in the end completely destroyed. In effective nodules the bacterial tissue continues to grow, so that on plants 10 weeks old its mean volume per nodule is 0.22 cu. mm. The nodule does not begin to show disintegration till it is nearly a month old and this process is not complete until it is about 8 weeks old, the organized bacterial tissue, formed by the end of the first week, having an active life of about 7 weeks. In ineffective nodules the bacterial tissues ceases to grow after about a week when its mean volume is about 0.05 cu. mm., and it is completely disintegrated by about the fifteenth day having thus an active life of little more than a week.

The cells of the bacterial tissue contain almost the entire bacterial population of the nodule, and this tissue, prior to its disintegration, may therefore be assumed to be the centre of nitrogen fixation. The activity of the nodule in fixing nitrogen must therefore be greatly affected by the differences above described, and should bear a relation to an integration of the volume of organized or undisintegrated bacterial tissue over the period covered by stages I and II.

D. RELATION OF VOLUME AND DURATION OF THE BACTERIAL TISSUE TO NITROGEN FIXATION IN CLOVER NODULES

A series of clover cultures in agar was made in order to obtain more exact data as to the changes in volume of organized and disintegrated bacterial tissue during nodule growth. The plants were grown in wide test tubes by the agar slope method above described. Half of the tubes were inoculated with the effective strain 2057 and half with the ineffective Coryn strain. The tubes were kept in a warm glasshouse and moisture maintained by the occasional addition of sterile culture solution.

Nodules were individually marked as they appeared, so that their age at the time of sampling might be known. Two tubes inoculated with each strain were taken at weekly intervals and the overall lengths of their nodules were recorded and measurements were made of the length and width of the organized and disintegrated bacterial tissue in these nodules, from free-hand sections, stained with 0.1% thionin made up in a 5% phenol solution. From these measurements the volumes of bacterial tissue were calculated, assuming a cylindrical shape. The results are shown graphically in figure 11, based on the examination of 152 nodules of strain 2057 and 71 Coryn nodules. If individual bacteria of the two strains are

equally active in fixing nitrogen in the nodule, the amount of nitrogen fixed by a nodule should be proportionate to the product, vt , of the mean volume of organized bacterial tissue and the time during which it acts. This product was obtained from the data by calculating the term $\{S(x)/n\}t$, where x represents the volume of organized bacterial tissue found on n

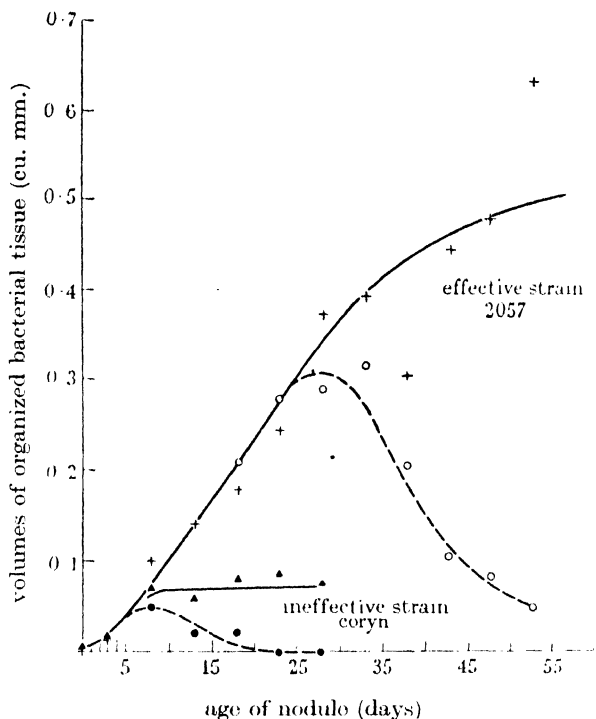


FIGURE 11. Changes in volume of total and of organized bacterial tissue in effective and ineffective clover nodules. ——— Total bacterial tissue; - - - organized bacterial tissue.

occasions over a growth period of t days. The calculation expresses the result in units of 1 cu. mm. bacterial tissue acting for 1 day ('cu. mm.-days').

The value of vt was found to be 8.25 cu. mm.-days per nodule for the effective strain 2057 and 0.42 for the ineffective Coryn strain. The difference in the factor vt should therefore account for a difference of nearly twenty-fold in the nitrogen fixed by individual nodules of the two strains. To determine the true nitrogen fixing efficiency of the bacteria of each strain it was thus necessary to estimate the amount of nitrogen fixed per unit volume of the organized bacterial tissue per day of each strain.

An experiment was made to determine the quantities of nitrogen fixed by red clover, grown in quart milk bottles of sand as described in the section on methods. The bottles were divided into three sets, one uninoculated, one inoculated with strain 205 and one with the Coryn strain. After a growth period of about 3 months, the number of nodules in each bottle was counted and the nitrogen determined by the Kjeldahl method. The results are set out in table 1. The amount of nitrogen fixed per bottle was

TABLE 1. NITROGEN-FIXING EFFICIENCY OF CLOVER BACTERIA
EXP. I, 1936

	Uninoculated	Strain Coryn	Strain 205
No. of replicate bottles	12	12	16
Nitrogen per bottle (mg.)	0.91 ± 0.136	2.42 ± 0.53	6.80
Nitrogen fixed per bottle (mg.)	—	1.51 ± 0.547	5.89
Mean volume \times duration of organized bacterial tissue per nodule in cu. mm. days (<i>vt</i>)		0.42	8.25
No. of nodules per bottle (<i>d</i>)		771	185
<i>e</i> = mg. nitrogen fixed per ml. active tissue per day = $1000f/vtd$		4.59	3.90

Applying $e = 3.90$ to data for Coryn.

Expected nitrogen fixed per bottle should be 1.27 mg.

obtained by subtracting the amounts found in the uninoculated set. The amount fixed per ml., per day, e , was calculated from the formula $e = 1000f/vtd$, where f is the nitrogen fixed and d the mean number of nodules per bottle.* The value of e for strain 205 is 3.9 mg. of nitrogen fixed per c.c.-day, and that for the Coryn strain is 4.59. If one assumes that the latter strain has the same efficiency as strain 205, a calculation can be made of the nitrogen that should be fixed per bottle by the Coryn strain if the value of e for this strain were also 3.9. This calculation gives the expected amount of nitrogen fixed per bottle as 1.27 mg. which does not differ significantly from the observed value of 1.51 whose standard error is ± 0.547 , with 11 degrees of freedom. Thus, although the plants with strain 205 nodules fixed nearly four times as much nitrogen as those bearing Coryn nodules, the whole of this difference can be accounted for by the smaller volume and shorter duration of the organized bacterial tissue in nodules of the ineffective strain.

* In this experiment, the data used for calculating volumes of bacterial tissue were derived from agar cultures but applied to the analytical results obtained from sand cultures. This procedure seemed to be justified because the mean lengths of 205 and Coryn nodules were in approximately the same ratio in the agar and sand cultures.

E. THE STRUCTURE OF PEA NODULES PRODUCED BY EFFECTIVE
AND INEFFECTIVE STRAINS

Observations were made on the anatomy of nodules produced by the effective strain 310 and by the ineffective strain B 33 on peas grown in small pots of sand. Roots were washed at different stages in the growth of the host plant, the nodules measured and sample nodules of various sizes were fixed and sectioned.

There was a marked difference in the size of nodules produced by the two strains. On plants 10 weeks old, strain 310 nodules of all ages had a mean length of 1.8 mm. and this included a number of long shaped and branched nodules. On plants of the same age, B 33 nodules had a mean length of 1.08 mm. and were spherical in shape.

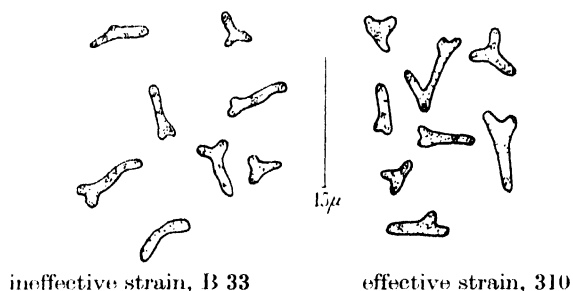


FIGURE 12. Bacteroid strains from pea nodules.

The course of nodule development in the pea generally resembles that in lucerne and clover. Sections showed that, on plants 10 weeks old, even large nodules of the 310 strain retained an active meristem cap and that very few showed any disintegration of the central tissue. Most of the nodules of B 33 strain, even on plants six weeks old, showed no active meristem cap and had their bacterial tissue completely disintegrated.

The appearance of the bacteria was particularly examined in view of the statements by Nobbe and Hiltner (1893) and Marie Löhnis (1930), that ineffective nodules contained bacteria in the rod stage which did not change into typical 'bacteroids'. In our material typical swollen and branched 'bacteroids' were found in the organized bacterial tissue of nodules produced by both the effective and ineffective strains (figure 12). These disappeared as usual during the process of disintegration of the bacterial tissue and so were absent in most nodules of the B 33 strain from plants over 6 weeks old, since by that time disintegration was usually complete.

Nobbe and Hiltner mention that their ineffective nodules showed no meristem cap and it therefore seems likely that they, at any rate, based their description on nodules in a state of disintegration, such as quickly follows the arrest of meristem activity. Marie Löhnis (1930) also describes certain unusual bacterial cells, referred to as 'brown bacteroids', in effective nodules. No such forms could be identified in our material, in which no constant differences in the shape of the bacterial cells could be found to distinguish the strains.

Young nodules produced by either strain contained considerable amounts of starch. Disintegrated bacterial tissue seldom contains any starch so that the earlier onset of disintegration caused an earlier disappearance of the starch in ineffective than in effective nodules.

Thus the principal differences found in effective and ineffective pea nodules are the smaller size of the latter and the shorter duration of the bacterial tissue in them. These differences are similar to those found in clover nodules.

F. THE COURSE OF GROWTH AND STRUCTURE OF SOY-BEAN NODULES PRODUCED BY EFFECTIVE AND INEFFECTIVE STRAINS

The material used for studying the anatomy of Soy bean nodules was obtained from sand cultures of the plants in large pots made in 1937, 1938 and 1939. The plants were inoculated with the effective strain 501 and with the ineffective strain 507, and nodules of various sizes were taken and fixed at different stages in the growth of the plant.

The development of nodules on the soy bean differs in some important respects from that in clover and pea nodules. Its general course has been described by Bieberdorf (1938) with whose account the present authors agree.

The nodules remain approximately spherical but become slightly flattened with age. Nodules of strain 501 continue to grow until they attain a considerable size. In the 1939 experiment, nodules of this strain, on plants four months old, attained a mean diameter, taken at right angles to the root, of 2.95 mm. Nodules of strain 507 stop growing quite early and remain small. The mean diameter of such nodules on four months old plants from the same experiment was only 1.9 mm. There is, in consequence, a large difference in volume of the bacterial tissue contained in the two types of nodule. Measurements were made of the bacterial tissue from freehand sections of 100 nodules of each strain taken from plants of varying ages grown in 1939. From these data the volumes were calculated, assuming

the shape of the bacterial tissue to be an ellipsoid. The volume of bacterial tissue is plotted against the overall diameter of the nodule in figure 7. The relation of the two characters differs in the two strains, an effective nodule of given length having more bacterial tissue than an ineffective one. The volume of bacterial tissue, corresponding to the mean diameters given above are, 11.4 cu. mm. for strain 501 and 2.3 cu. mm. for strain 507.

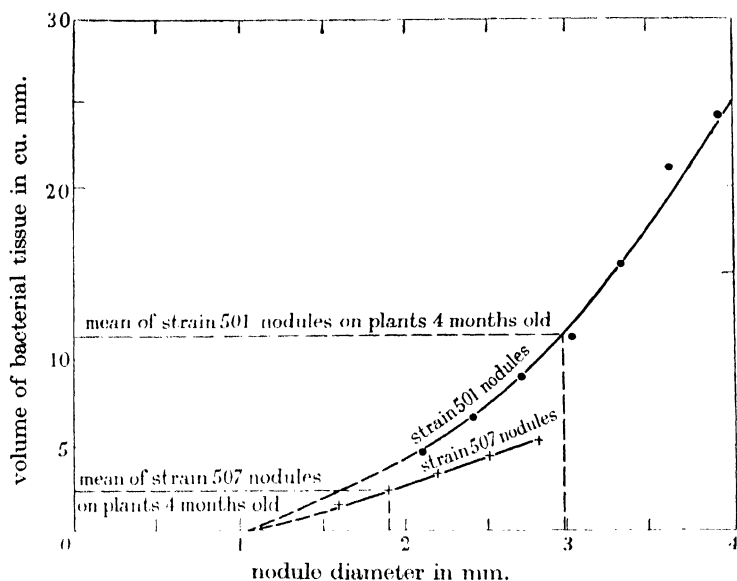


FIGURE 13. Relation of overall diameter to volume of bacterial tissue in soy-bean nodules. Each point gives the mean volume of bacterial tissue in from three to thirty-two nodules of the same overall length.

The bacterial tissue of soy-bean nodules differs in appearance from that of clover and pea nodules. In the latter the great majority of cells are filled with bacteria both in effective and ineffective nodules, so that the volumes of this tissue in the two types gives a comparable estimate of the relative numbers of bacteria present. But in soy-bean nodules the bacterial tissue contains a relatively large proportion of uninfected cells and this proportion differs strikingly in effective and ineffective nodules studied (figures 14, 15, plate 14). Estimates of the percentage volumes of infected cells in the bacterial tissue were obtained by measuring the areas occupied by infected and by uninfected cells in random microscope fields in microtome sections from a number of nodules of each type. The results are shown in table 2. Measurements made from soy-bean nodules grown in 1938 showed that 82.99% of the volume was occupied by infected cells in strain 501

nodules and 43.44% in strain 507 nodules. In the material grown in 1939, the percentages were 94.19 for strain 501 and 49.16 for strain 507. The percentages for the two strains in each year were in the same ratio, namely 1.91 : 1, but they were different in the two seasons, this seasonal difference being significant in the case of strain 501. In the infected cells of both types of nodules, the bacteria lie close-packed in the cytoplasm, so that a comparison of the percentage volumes of infected cells should provide an estimate of the relative numbers of bacteria per unit mass of the bacterial tissue in the two types of nodule.

TABLE 2

	501 nodules		507 nodules	
	1938	1939	1938	1939
No. of nodules examined	5	11	5	11
Percentage volume of infected cells (mean)	82.99	94.19	43.44	49.16
Standard error	± 3.15	± 1.05	± 5.19	± 2.05

In soy-bean nodules the infected cells were found to be free from starch, but the uninfected cells contained many starch grains. Since these latter cells were much the more numerous in inefficient nodules, the amount of starch in these nodules was proportionately greater. The 507 nodules somewhat resembled the ineffective *Phaseolus* nodules, described and illustrated by Elizabeth McCoy (1929), in having many uninfected cells filled with starch in the central tissue.

The bacteria in infected cells in soy-bean nodules remain in the rod stage and show scarcely any change toward the 'bacteroid' condition in any part of the nodule. There was no noticeable difference in their appearance in the organized bacterial tissue in nodules produced by the two strains.

The process of disintegration of the bacterial tissue in soy beans differs in important respects from that in clover and pea nodules. It does not always begin at the base but may start at any point in the central tissue. In the early stages, the bacteria break up into granules and the infected host cells lose their turgidity. The uninfected cells, however, remain turgid and their pressure on the infected cells causes these to collapse. When this process is far advanced the appearance of a section suggests, and was, indeed, at first mistaken for intercellular infection (figure 16, plate 14). It probably accounts for the statement of Kás (1930) that soy-bean nodules show both intra- and intercellular infection, and may also explain the 'intercellular' type of infection described in *Serradella* by Milovidov

(1926, 1928). Bacterial tissue showing this collapse of the infected cells is here referred to for convenience as 'disintegrated', although there is no general collapse of the tissue as occurs in clover and pea nodules. This is clearly due to the large proportion of uninfected cells which remain turgid, and support the tissue.

There is a marked difference between nodules of strains 501 and 507 in the age at which disintegration takes place. In the 1939 nodules of strain 507 the process began when the host plant was only 4 weeks old and was nearly complete by the twelfth week. Strain 501 nodules on plants twelve weeks old did not yet show any disintegration, which only began to appear when the plants were at the end of their growth period, at an age of about 17 weeks.

Effective and ineffective soy-bean nodules thus show differences in their growth and decay of the same general type as those found in clover and pea nodules. Effective nodules grow to a mean diameter 50 % greater than that of ineffective nodules. They contain more than five times the volume of bacterial tissue, which bears twice the percentage by volume of infected cells, and this tissue lasts more than four times as long as in ineffective nodules before becoming disintegrated.

It was, however, necessary to follow the course of growth and decay of the bacterial tissue throughout the growing period of the plant before the quantitative effect of these factors on nitrogen fixation could be estimated.

The size of the plant made it impracticable to use the agar culture method, used with clover for obtaining individual nodules of known age. The measurements here recorded are therefore the means from batches of nodules taken from successive reapings of plants of recorded ages taken during the pot-culture experiment of 1939. These data did in fact give a good measure of the growth and decay of the nodules because, as is usual in soy beans, nearly all the nodules appeared when the plants were quite young and so did not vary greatly in age within each batch.

The soy beans were grown in thirty large pots, twelve of which were inoculated with strain 501, twelve with strain 507, while six were left uninoculated. Three or four plants inoculated with each strain were removed at weekly intervals, and, from these, twenty nodules of each type were examined by means of hand sections stained with carbol thionin. In each section, measurements were made of the diameters of the nodules and of the area occupied by bacterial tissue, and an estimate was made of the percentage of that area showing disintegration. From these measurements, the mean volumes of organized and of disintegrated bacterial tissue in each batch of twenty nodules were calculated. The volumes of total (that is,

organized plus disintegrated), bacterial tissue have been plotted against nodule diameter in figure 13, already discussed. Changes in volume of the organized bacterial tissue, with time are shown in figure 7, and discussed below. At the end of the growth period, the remaining plants, then 4 months old, were reaped, the nodules counted and nitrogen determinations made by the Kjeldahl method.

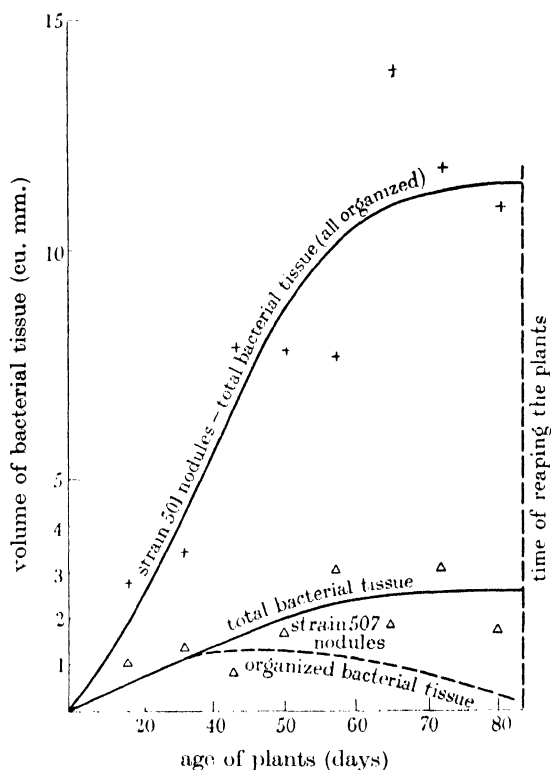


FIGURE 17. Changes in volume of organized and total bacterial tissue in effective and ineffective soy bean nodules. Each point represents the mean of 20 nodules.

In figure 17, the calculated volumes of total and of organized bacterial tissues, taken from the weekly measurements, are plotted against the age of the plants. From these data the integrated cu. mm.-days, vt , are calculated according to the formula

$$vt = \frac{S(b - by)}{n100} t,$$

where b is the mean volume of total bacterial tissue found on each occasion and y the estimated percentage of that tissue showing disintegration.

The values of vt thus calculated, are 83.95 for strain 507 and 647.4 for strain 501. These are in units of 1 cu. mm. of *organized* bacterial tissue acting for one day, but they do not represent the relative volumes of *active* tissue until further corrected for the percentage, c , of infected cells within the bacterial tissue of each strain. When this correction is made, using the mean percentages obtained from 1939 data shown in table 2, the value of $vtc/100$ for strain 507 is 41.27 and for strain 501 it is 609.79. The average 501 nodule should thus fix nearly fifteen times as much nitrogen as the average nodule of strain 507, by reason of its larger content of infected cells.

The data with regard to nitrogen fixation are set out in table 3. Plants with 501 nodules fixed about six times as much nitrogen as did those with 507 nodules. The quantity e of nitrogen fixed per ml. of active infected cells per day was calculated from the formula $e = 1000f / vt \frac{c}{100} d$ where f and d are means per plant for nitrogen fixed and nodule numbers respectively.

TABLE 3. NITROGEN-FIXING EFFICIENCY OF SOY-BEAN BACTERIA
(1939 EXPERIMENT)

	Uninoculated	Strain 507	Strain 501
No. of plants	40	33	18
Nitrogen per plant (mg.)	16.69	19.34	31.70
Nitrogen fixed per plant (mg.)		2.65	15.01
Mean volume \times duration of organized bacterial tissue per nodule in cu.mm.-days (vt)		83.95	647.4
Infected cells as percentage of organized bacterial tissue (c)		49.16	94.19
No. of nodules per plant (d)		45.2	21.1
$e =$ mg. nitrogen fixed per c.c. active infected cells per day $= 1000f / vt \frac{c}{100} d$		1.42	1.17

Applying $c = 1.17$ to data of strain 507.

Expected nitrogen fixed per plant should be 2.18.

The value of e , for the two strains 507 and 501, were 1.42 and 1.17 respectively. If the value for strain 507 were the same as for the other strain, namely 1.17, the expected nitrogen fixed per plant would be 2.18 mg., actually a fraction less than the observed figure. Thus the whole of the large difference in nitrogen fixed by soy-bean plants bearing the two types of nodules can be accounted for by differences in the total volume of infected cells and in the duration of their activity.

DISCUSSION

The course of the growth and decay of nodules produced by effective and ineffective strains of bacteria on clover, peas and soy beans thus shows that there are two quantitative characters closely correlated with effectiveness, namely the combined volume of the infected cells and the length of time that elapses before they collapse and disintegrate. Other features of nodule anatomy, unless causally connected with the above two characters, were found to show no association with effectiveness. Attempts were made with clover and soy beans to give quantitative values to these factors of volume and duration of the organized tissue containing bacteria, and to apply these values as corrections in estimating, from the results of analysis, the quantities of nitrogen fixed by a unit volume of infected cells in unit time. These attempts were so far successful that in experiments with both host plants, the whole of the large differences between the amounts of nitrogen fixed by 'effective' and 'ineffective' strains respectively could be accounted for by the differences in volume and duration of the infected nodule cells. The data showed no evidence that the 'ineffective' strains were really less efficient in fixing nitrogen per unit of bacterial mass in unit time, than the 'effective' strains.

The problem of ineffective nodules thus resolves itself into the need for explaining why the volume of infected cells in them remains so small and why these cells disintegrate so soon. The small volume of bacterial tissue is related to the early cessation of apical meristem growth in the nodule, but since this meristem activity is doubtless stimulated by the bacteria, both the short growth period of the nodule and the early decay of the infected cells are indications of poor bacterial growth within the tissues.

Strains of nodule bacteria differ in the rapidity of their growth on artificial media, but there is no correlation between this character and their effectiveness in the host plant. There is thus no reason to believe that ineffective strains are naturally less vigorous in growth than others, when given a suitable medium. Hence the poor growth of these strains within the nodule indicates that the tissues of the host plant provide an environment that is less suited to the ineffective than to the effective strains.

This may either mean that some unfavourable factor is normally present in the host tissues, or that such a factor appears in them as a consequence of infection by the ineffective strain. The latter alternative is rather suggested by the fact that ineffective nodules commence their growth quite normally and only later show arrested development.

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DESCRIPTION OF PLATES

Plate 13

Figures 1 to 3. Development of an ineffective clover nodule produced by the Coryn strain.

FIGURE 1. Nodule 1 day old, composed of meristem tissue.

FIGURE 2. Nodule 3 days old, showing swelling of the central tissue cells, and formation of a vascular strand.

FIGURE 3. Nodule 6 days old, and about fully grown, showing the small amount of bacterial tissue.

FIGURE 5. Effective clover nodule one month old produced by strain 205, showing large development of bacterial tissue.

FIGURE 8. Six weeks old nodule produced by strain 205. Disintegration of the bacterial tissue commencing at the base.

m.c. meristem cap; *o.b.t.* organized bacterial tissue; *d.b.t.* disintegrated bacterial tissue; *v.s.* vascular strands; *r.s.* stele of root.

Plate 14

FIGURE 9. Clover nodule, 15 days old, produced by the Coryn strain, showing disintegration of the bacterial tissue. *d.b.t.* disintegrated bacterial tissue; *v.s.* vascular strands; *r.s.* stele of root.

Figures 14 to 16. Sections of the bacterial tissue of soy-bean nodules.

FIGURE 14. Strain 501 (effective), showing large proportion of infected cells, *i.e.*

FIGURE 15. Strain 507 (ineffective), showing small numbers of infected cells and large proportion of uninfected cells, *s.c.* containing starch.

FIGURE 16. Bacterial tissue of strain 507 nodule undergoing disintegration through collapse of the infected cells, *i.e.*, the sterile cells, *s.c.*, remaining turgid.

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The growth of nodule bacteria in the expressed juices from legume roots bearing effective and ineffective nodules

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Strains of pea and soy-bean nodule bacteria, differing in their effectiveness in benefiting the host legume, were grown in media containing the unheated root juices from uninoculated host plants and from host plants bearing effective and 'ineffective' nodules, and their growth was measured.

The growth of the different bacterial strains on root juice from uninoculated plants was not correlated with their effectiveness.

The juice from roots with effective nodules produced significantly better growth of the bacteria than juice from roots with ineffective nodules in twenty-seven comparisons out of forty-four, the differences in the remaining comparisons being insignificant.

The juice from roots with effective nodules produced significantly better growth than the juice from uninoculated roots in ten comparisons out of twenty-five, and significantly poorer growth in three comparisons.

The juice from roots with ineffective nodules produced significantly poorer growth than the juice from uninoculated plants in eleven comparisons out of twenty-five, and better growth in only one comparison.

The production, as a result of infection, of soluble substances affecting growth of the bacteria, affords an explanation of those differences in nodule growth that determine the effectiveness or ineffectiveness of the different strains of bacteria as regards nitrogen fixation within the host.

INTRODUCTION

Different strains of bacteria-producing nodules on a given host legume differ greatly in their ability to benefit that host by fixing nitrogen. Some strains are so 'ineffective' that they produce no appreciable improvement in growth of the legume and fix nitrogen in amounts difficult to detect.

The ineffectiveness of such strains was at first attributed to some defect in the process of nitrogen fixation.

The recent study of clover, pea and soy-bean nodules made by Thornton and Chen (1940), however, has shown that nodules produced by an ineffective strain contain a much smaller volume of infected cells filled with nodule bacteria than do effective nodules, and also that in them, the central tissue containing these infected cells disintegrates after a relatively very

short active life. Estimates were made of the mean volume and duration of this central 'bacterial tissue' in nodules of effective and ineffective types. Plants of both clover and soy beans showed large differences in nitrogen content according to which type of nodule they carried, but the quantities of nitrogen fixed by a unit mass of infected nodule cells in unit time was found to be the same for effective and ineffective nodules. The whole of the large differences in total nitrogen fixed could be accounted for by differences in the total volume of infected nodule cells and the duration of these cells prior to disintegration. The further elucidation of the cause of ineffectiveness thus requires that the relatively small total volume and the short life of the infected cells in ineffective nodules be explained.

Nodules produced by effective and ineffective strains develop during their early stages in the same manner, and begin to grow at the same rate. But after a very short while (with clover in about 7 days), the ineffective type of nodule stops growing, while nodules produced by an effective strain continue their growth. This arrested growth is the principal cause of the small volume of infected cells contained in an ineffective nodule, although in soy beans the smaller proportion of infected to sterile cells in the central tissue is a contributory factor. Arrested growth is due to the stopping of cell division in the apical cap of nodule cells. Presumably the initiation and maintenance of the apical meristem is due to growth substances produced by the bacteria. The production of growth substance by the nodule bacteria has been demonstrated by Link (1937) and Thimann (1936, 1939), but Chen (1938) found that effective and ineffective strains of clover bacteria when growing equally vigorously in liquid culture produced similar amounts of growth substance, as assayed by Went's split pea stem test. The stopping of growth in an ineffective nodule would thus seem to be a consequence of the early arrest or decrease in growth of the contained bacteria. Weaker growth of the invading bacteria in ineffective nodules is also suggested by the observation that in soy-bean nodules the central tissue contains a smaller proportion of infected cells in ineffective than in effective nodules.

There is considerable variation in the growth rates of various strains of nodule bacteria in laboratory media, but no correlation could be found between this character and effectiveness towards the host plant. So that the striking difference between the growth of effective and ineffective strains of bacteria within the nodule is not due to any inherent difference in their powers of multiplication, but must indicate that the latter type of strain finds the host tissues an unsuitable environment for its growth.

Disintegration of the central bacterial tissue with its contained bacteria seems eventually to take place in all nodules by whatever strain they are produced, although in effective nodules it does not normally occur until the nodule is getting old. It can, however, be induced in quite young nodules, even of effective strains, by imposing abnormal conditions on the host plants, as by growing these in a boron-deficient medium (Brenchley and Thornton 1925), in the dark (Thornton 1929), or with an excessive nitrate supply (Thornton and Rudolf 1936). In nodules produced by ineffective strains on normally-grown host plants, the disintegration of the central tissue and bacteria also takes place in quite young nodules, and is closely similar to that which can be produced by artificially changing the physiology of the host plant. Like the stopping of nodule growth, the disintegration indicates a maladaptation between host plant and nodule organism.

This maladaptation of inefficient strains to their host plant may be due to any of the following three causes: (a) Some factor may be normally present, in the host root system, that is specifically less favourable to the ineffective than to the effective strains. (b) The presence of an effective strain within the tissues may induce a change favourable to the growth of the bacteria, which is not induced by the presence of the ineffective strain. Here the action of the induced factor need not specifically favour either type of strain. (c) The presence of invading bacteria of an ineffective strain may induce some change in the host tissue that is detrimental to growth of the bacteria. Here again the induced factor need not act specifically against ineffective strains, but these strains must be the specific cause of its appearance.

The substances in solution within the nodule tissue must supply food material to the bacteria so that it seemed natural to search in the root and nodule juice for the factors affecting their growth. On our first hypothesis, (a), the root juice of the host plant, whether uninoculated or bearing either type of nodule should be specifically less favourable to the growth of ineffective than to effective strains of the bacteria. On the second hypothesis, (b), juice from roots bearing effective nodules should be more favourable to the growth of the bacteria than that from roots bearing ineffective nodules or from uninoculated roots. On the third hypothesis, (c), juice from roots bearing nodules containing an ineffective strain should be less favourable to the growth than juice from roots bearing effective nodules or from uninoculated roots.

To test these hypotheses, plants were grown in sterilized sand without inoculation, and inoculated with effective and ineffective strains; the root

juices were extracted from each set, sterilized by filtration and their effects on the growth of nodule bacteria of either type were tested *in vitro*. Clover was found to be unsuitable for this work owing to its small root development in the young state. Peas and soy beans were used because these provide ample root material, and since strains showing wide differences in effectiveness have been isolated from their nodules (Helz, Baldwin and Fred 1927; Wright 1925). The cultures of pea and soy-bean nodule bacteria used in this work were supplied by the Wisconsin Agricultural Experiment Station, to whom the authors are gratefully indebted.

TECHNIQUE

The plants were grown at Rothamsted in sand in glazed earthenware pots, the peas in small pots holding 3 kg., and the soy beans in large pots holding 12 kg. of sand. In the experiments with peas, the sand was rendered free from live nodule bacteria by blowing superheated steam through the pots of sand via the aperture at the base. The sand used for experiments with soy beans was not sterilized, since it was found not to contain bacteria capable of producing nodules on soy beans. In all the experiments described below, whether with peas or soy beans, the uninoculated sets remained free from nodules.

In the experiments carried out in 1936 and 1937 the following food solution was added to the sand, to about 15% of its dry weight, and fresh supplies were given during the growth of the plant:

K_2SO_4	0.87 g.	$CaSO_4$	0.2 g.
K_2HPO_4	0.3 g.	$FeCl_3$	0.04 g.
KH_2PO_4	0.3 g.	$MnSO_4$	0.04 g.
NaCl	0.5 g.	Boric acid	0.04 g.
$MgSO_4 \cdot 7H_2O$	0.5 g.	Tap water	1 litre

For the 1939 experiments this food solution was modified as shown below, so as to give a better calcium supply.

K_2SO_4	0.9 g.	$FeCl_3$	0.04 g.
K_2HPO_4	0.5 g.	$MnSO_4$	0.04 g.
$CaH_2(PO_4)_2 \cdot 4H_2O$	0.5 g.	Boric acid	0.04 g.
$MgSO_4 \cdot 7H_2O$	0.5 g.	Tap water	1 litre
NaCl	0.5 g.		

The food solutions were sterilized before addition to the pots, and in the experiments with peas all watering was with boiled water.

In all experiments, inoculation was accomplished by mixing a suspension of the appropriate organism with the food solution before this was poured into the sand, the uninoculated pots receiving an equal volume of sterile water in place of the bacterial suspension. The seeds were externally sterilized by immersion in absolute alcohol followed by 0.2% aqueous HgCl_2 for 3 min., and then washed in four changes of sterile water. They were sown immediately after application of the food solution to the pots. When the plants reached the flowering stage, the roots were shaken free from sand, washed, and the surplus water drained off them. They were then minced, ground in a mortar and the juice squeezed through muslin. This juice was then passed through two thicknesses of Whatman no. 4 filter-paper, and further cleared by filtration under reduced pressure through a pad 2 in. thick of compressed filter-paper fragments set up in a wide glass cylinder.* To prepare this pad, wet pieces of filter-paper about 1 cm. square were compressed with a glass ramrod. In the 1937 experiment with soy beans this filter was replaced by a Seitz filter.

After clearing, the juice was rendered sterile by filtration successively through an L1 Chamberland filter-candle, and through a sterilized L5 candle set up in a sterile vacuum flask.

The effect of the various root juices upon the growth of the nodule bacteria *in vitro* was tested by two different methods. In the 1936 experiments with soy beans and peas the juices were added to a melted agar medium which was poured into petri dishes and allowed to set. A number of point inoculations of the test organism were made on each plate, and the mean area of the resulting colonies was taken as the measure of growth.

In later experiments the test organisms were grown in liquid media containing the root juices, and their growth was measured by haemocytometer counts. As the details of technique varied somewhat in the different experiments they will be described with each experiment.

EXPERIMENT WITH SOY BEANS, 1936

Five sets of soy beans were grown in pots of sand. One set was uninoculated and the remaining sets inoculated with nodule bacteria of the effective strains 501 and 505, and of the ineffective strains 502 and 507. Six parallel pots, each with eight plants, were set up and growth carried out from 1 September till 26 October.

* The apparatus used is illustrated in *Proc. Roy. Soc. B* (1936), 119, figure 1, p. 478.

The roots were washed and the juice extracted and sterilized by filtration. The medium used for testing the effects of the juices on bacterial growth had the following composition:

K_2HPO_4	1.0 g.	$FeCl_3$	0.02 g.
KH_2PO_4	1.0 g.	Sucrose	10.0 g.
$MgSO_4 \cdot 7H_2O$	0.4 g.	Yeast water*	100 ml.
NaCl	0.2 g.	Tap water	900 ml.
$CaCO_3$	1.0 g.	Agar	20.0 g.

This basal medium was divided into five portions which were autoclaved, cooled to 42° C and, to each portion, one of the juices was added with a sterile pipette to make 18 % of the final volume. From each of the four media containing juice from the inoculated plants, sixteen petri dish plates were poured, each of 10 ml. of medium. These were allowed to set and incubated at 25° C for 24 hr. to allow the agar surface to dry. Each batch of sixteen plates were then divided into four sets of four replicate plates, one set being inoculated with each of the four strains of soy-bean nodule organisms 501, 505, 502 and 507.

In the case of the uninoculated roots, however, the small supply of juice enabled only three replicate platings to be inoculated with each test organism.

By the above plan each of the four strains was grown on media containing each of the five juices, namely, from uninoculated plants and from plants that had borne nodules produced by each of the same four strains (see table 1).

The inoculation of the agar plates was carried out as follows. Ten needles were set about 1.5 cm. apart in a large cork, with their points projecting and carefully adjusted so that the points were level. The culture to be used as inoculum was suspended in sterile saline solution, and about 10 ml. were poured into a sterile petri dish. The needles, sterilized by flaming, were lowered into the suspension, and their points then gently brought down on to the surface of the agar medium in the plate. In this way each plate was given ten point inoculations. After incubation for 14 days at 25° C, the colony areas were measured. The results are shown in table 1, which gives the mean colony areas in square millimetres. The mean colony area on each plate was taken as the unit in calculating the standard error. The number of degrees of freedom for each set is given in the table. This is not always three, because less than four replicate plates with juice from

* Yeast water was made by boiling 10 % yeast in water and filtering.

uninoculated plants were tested with each strain, and because some plates in other sets were lost from various causes. In particular, the set in which strain 502 was tested on juice 501 was spoilt owing to water condensing on the agar surface. In order to obtain a valid standard error for the whole experiment, a hypothetical value was allotted to this set, calculated by Yates's method for missing plots (Yates 1933). The standard error for the differences between any two individual set means is ± 1.38 . The bottom row gives the mean colony areas for the four test organisms combined in the case of each juice tested. The standard error for the differences between any two of these juice means is ± 0.69 .

TABLE 1. EXPERIMENT WITH SOY BEANS, 1936

Colony areas of four strains of soy-bean nodule bacteria on agar media containing root juice from variously treated plants

Strain	Mean colony area sq. mm. (<i>m</i>)	Medium containing root juices from				
	Degrees of freedom	Uninoculated plants (control juice)	Plants inoculated with strain			
	(<i>n</i>)		501	505	502	507
501	<i>m</i>	4.71	6.75	11.35	0	0
	<i>n</i>	1	3	3	3	3
505	<i>m</i>	8.58	6.06	14.04	6.27	8.17
	<i>n</i>	2	3	3	2	2
502	<i>m</i>	8.49	(11.52)	19.56	6.22	10.96
	<i>n</i>	1	0	4	3	2
507	<i>m</i>	6.28	7.07	7.99	5.18	4.37
	<i>n</i>	0	3	2	3	3
All strains	<i>m</i>	7.02	7.85	13.23	4.41	5.87

S.E. of difference between any two individual means = ± 1.38 .

S.E. of differences between juice means (bottom row) = ± 0.69 .

The relative effect on the bacterial growth of root juice from plants bearing effective nodules, as against root juice from plants with ineffective nodules, can be tested in fourteen comparisons, excluding, of course, the hypothetical value. Juice from plants bearing the effective strain gave significantly the better growth in ten of these comparisons.

The root juice from plants bearing 501 nodules did not differ significantly from the juice of uninoculated roots (described below as 'control juice') in its effects on any of the strains tested. Root juice from plants bearing the other effective strain 505, however, gave significantly better growth than did control juice with three of the test organisms and no significant difference with the fourth.

Juice from roots bearing ineffective nodules of strains 502 and 507, completely prevented growth of strain 501, but with the other three test organisms these two juices produced growth not significantly different from control.

In this experiment the results are suggestive, but the difference in action of the two juices from plants with effective nodules and the different response of the four test organisms to the juices from plants with ineffective nodules, makes the comparison with control juice inconclusive.

EXPERIMENT WITH PEAS, 1936

In this experiment peas, inoculated with the effective strains 310 and 317, with the ineffective strains 313 and B33 and uninoculated, were grown in small pots of sand as described above. The seeds were sown on 1 September and the plants reaped on 21 October. The root juices were extracted, and the growth of the four strains of pea-nodule bacteria on agar media containing each of the juices was tested by the same method as in the previous experiment. Each organism was, in addition, grown on the basal agar medium in which the root juice was replaced by distilled water. The results are shown in table 2 in which the mean colony areas are derived from three to five replicate plates, each bearing ten colonies. The standard error for the difference between any two individual means is ± 18.38 , while that for the difference between the juice means given in the bottom row is ± 9.19 .

In this experiment, juice from plants with efficient nodules gave significantly better growth than juice from plants with inefficient nodules in eight out of the sixteen possible comparisons and insignificant results in the remainder.

In comparison with the uninoculated control juice, that from roots bearing one of the effective strains (310), has given variable results, and its mean effect on all four test organisms does not differ significantly from that of the control juice. Roots bearing the other effective strain (317) have produced a juice giving significantly better growth of two test organisms, as compared with control juice, but insignificant results with the other two. Both the root juices from plants bearing ineffective nodules (strains 313, B33) produced poorer growth than control juice with every test organism, although these differences were significant in only three out of the eight comparisons with individual test organisms. But when the growths of the four test organisms are combined, the growth on these two

juices differs from that on control juice with a high degree of significance. In only one comparison out of the eight did these two juices produce significantly better growth than the medium without any root juice at all.

TABLE 2. EXPERIMENT WITH PEAS, 1936

Colony areas of four strains of pea-nodule bacteria on agar media with and without root juices

Strain	Mean colony area	Medium containing root juices from					Medium without root juices
	sq. mm.	Uninoculated plants (control juice)					
	(<i>m</i>)		Plants with nodules by strain				
	Degrees of freedom (<i>n</i>)		310	317	313	B 33	
310	<i>m</i>	153.4	198.6	190.2	106.3	151.3	102.5
	<i>n</i>	3	3	3	3	4	4
317	<i>m</i>	159.4	114.2	145.3	104.3	112.2	94.0
	<i>n</i>	2	3	3	3	2	3
313	<i>m</i>	105.6	103.3	156.7	92.5	94.3	90.0
	<i>n</i>	2	3	3	3	3	4
B 33	<i>m</i>	130.6	124.1	148.1	94.7	113.6	112.6
	<i>n</i>	3	3	3	3	3	3
All strains	<i>m</i>	137.3	135.0	162.3	94.4	117.8	99.7

s.e. of differences between individual means = ± 18.38 .

s.e. of differences between juice means = ± 9.19 .

This experiment thus confirms the conclusions of the first experiment in that plants bearing one of the effective strains (317) produced a juice stimulating bacterial growth compared with control juice, while the juice from plants bearing either of the ineffective strains was definitely less good than control juice as a medium for the growth of the test bacteria.

EXPERIMENT WITH PEAS, 1937

The method of estimating bacterial growth by colony area was found to be not altogether satisfactory owing to uneven growth of the colonies; it was, therefore, decided to estimate growth by means of haemocytometer counts of the test organisms growing in liquid culture.

Peas were grown in small pots of sand, sets of eight replicate pots being inoculated with strains 310, 317 and B 33, and one set left uninoculated. The peas were grown from 8 March till 6 June, and the root juices extracted

and sterilized by filtration. The test organisms were grown in the following liquid medium:

K_2HPO_4	0.5 g.	$CaCO_3$	0.5 g.
KH_2PO_4	0.5 g.	Sucrose	10.0 g.
$MgSO_4 \cdot 7H_2O$	0.2 g.	Yeast water (10 % yeast)	100 ml.
NaCl	0.1 g.	Tap water	900 ml.
$CaSO_4$	1.0 g.		

The medium was put up in 20 ml. portions in small flasks, sterilized in the autoclave and 5 ml. of juice added aseptically to each flask. Duplicate flasks of medium containing each type of juice were inoculated with standard loopfuls of a suspension of B33 strain of pea-nodule bacteria. After 18 days' growth at 25° C, the numbers of bacterial cells were counted on a haemocytometer ruled in $\frac{1}{400}$ sq. mm. The results are set out in table 3, where each value given is the mean count of duplicate flasks. The standard error for the difference between any two means is ± 25.9 .

TABLE 3. EXPERIMENT WITH PEAS, 1937

Growth of pea organism, strain B33. Bacterial cells per ml. in liquid media containing root juices, means of duplicate cultures

Medium containing root juices from peas			
Uninoculated (control juice)	Inoculated strain 310	Inoculated strain 317	Inoculated strain B33
1828	2083	1915	1608
s.e. of difference between any two means = ± 25.9 , $n = 4$			

Both the efficient strain root juices gave significantly better growth than did the control juice. The juice from roots bearing the inefficient B33 nodules produced less growth than any of the other juices, the significance of the differences having in each case a probability of less than 1 %.

EXPERIMENT WITH SOY BEANS, 1937

Soy beans were grown in large pots with one set uninoculated and other sets inoculated with strains 501, 502, 505 and 507. The seed was sown on 10 April and the plants reaped on 10 July. Root juices were extracted and sterilized as before. A liquid medium was made up similar to that used in the last experiment but with the sucrose replaced by dextrose, which was found better for the growth of soy-bean bacteria. This medium was put up

in 15 ml. portions in wide test-tubes, to each of which, after sterilization, 5 ml. of juice to be tested was added aseptically. Batches of six replicate tubes were given control juice, and juices from plants bearing 505 and 507 nodules respectively. Two of each of these tubes were inoculated with strain 501, two with strain 502 and two with strain 507. The yield of juice from plants bearing 501 and 502 nodules was insufficient, so that only two strains of test organisms, 501 and 502, were grown on these juices, each in duplicate tubes.

After 12 days' incubation at 25° C, the growth of the bacteria was estimated from haemocytometer counts. The results of this experiment are shown in table 4.

TABLE 4. EXPERIMENT WITH SOY BEAN, 1937

Growth of soy-bean organisms. Bacterial cells per ml. in liquid media with root juices, mean of duplicate cultures

Test organism	Uninoculated (control juice)	Medium containing root juice from soy beans			
		Inoculated with strain			
		501	505	502	507
507	1251	—	790	—	668
s.e. of difference between any two means, 65.1, $n=3$					
501	1835	1938	1533	403	960
502	1680	2122	1263	1328	660
Means of both strains	1757	2030	1397	866	810

s.e. of the difference between individual means = ± 210.7 , $n=10$

On account of the incompleteness of the test with 507 bacteria, separate standard errors have been calculated for this series and for the remaining two series inoculated with 501 and 502 bacteria. With the former, the standard error for a difference between the means is ± 65.1 , while for the latter the error for differences between individual means is ± 210.7 .

When growth on juices from plants bearing efficient and inefficient strains is compared for the three test organisms, the inefficient strain juices give significantly the poorer growth in seven, and insignificant differences in two of the nine comparisons.

Five comparisons can be made between growth of the test organisms on juices from plants bearing effective nodules of strains 501 and 505 with their growth on juice from uninoculated plants. In one comparison the former type of juice gave significantly better and, in a second, significantly poorer growth. The other differences were not significant. The two root

juices from plants bearing inefficient nodules of strains 502 and 507 gave significantly poorer growth than did the control juice in four comparisons out of five, and gave an insignificant result in the remaining comparison.

This experiment thus confirmed the depressing action of juices from roots with inefficient strains, but did not bear out the previous experiments as regards the improved growth on juices from roots with the efficient nodules as compared with control juice.

EXPERIMENT WITH PEAS, 1939

Peas were grown in small pots of sand with one set uninoculated and others inoculated with strains 317 and 313. The plants were grown from 4 April till 26 June, and the root juices extracted and filtered as before. Small flasks were set up, each containing 50 ml. of the following medium in which the yeast extract was replaced by asparagin as a nitrogen source, so that the root juice should supply the sole source of accessory growth substances.

K_2HPO_4	0.5 g.	$CaSO_4$	0.5 g.
KH_2PO_4	0.5 g.	$FeCl_3$	0.02 g.
$MgSO_4 \cdot 7H_2O$	0.2 g.	Asparagin	0.5 g.
NaCl	0.1 g.	Dextrose	5.0 g.
$CaCl_2$	0.1 g.	Tap water	1000 ml.

The flasks of medium were sterilized, and to each 10 ml. of root juice were added aseptically. Each flask was inoculated with 1 ml. of a suspension of the test organism. Eight flasks of control juice and six of each of the other types of juice were set up, and half the flasks with each juice were inoculated with 317 and half with B33 organisms. Two of the flasks inoculated with strain B33 and one inoculated with strain 317 were lost by contamination, so that the number of replicates counted varies between the different sets as shown in table 5. The results of the haemocytometer counts after 10 days' incubation at 25° C are shown in table 5.

The growth of strain 317 was poor on all media, and did not make significantly different growth on any of them. Organism B33 grew better. Its growth was much poorer on juice from roots bearing inefficient 313 nodules than on control juice, but growth was also significantly poorer on the 317 juice. Indeed, the two juices from inoculated roots did not differ significantly from each other in their effects in this experiment.

TABLE 5. EXPERIMENT WITH PEAS, 1939

Growth of pea-nodule bacteria, strains 317 and B33. Bacterial cells per ml. in media with pea-root juices

Test organisms	Bacterial numbers (<i>m</i>) Degrees of freedom (<i>n</i>)	Media containing root juices from peas		
		Uninoculated (control juice)	Inoculated with strain	
			317	313
317	<i>m</i>	169	145	156
	<i>n</i>	3	2	2
s.e. of difference between two means = ± 12.73 , $n = 7$				
B 33	<i>m</i>	2079	1516	1295
	<i>n</i>	2	1	2
s.e. of difference between two means = ± 129.8 , $n = 5$				

EXPERIMENT WITH SOY BEANS, 1939

Soy beans were grown in large pots of sand with one set uninoculated and other sets inoculated with strains 501 and 507 respectively. The seed was sown on 4 April and after 10 weeks' growth the roots were washed and the root juices extracted and sterilized by filtration. The same liquid test medium was employed as in the last experiment with peas; duplicate flasks were set up with each type of juice, 10 ml. of juice being added aseptically to 50 ml. of sterilized medium. All the flasks were inoculated with 1 ml. of a suspension of strain 507 and incubated at 25° C for 3 weeks, growth of the bacteria being slow. Haemocytometer counts of the resulting growth gave the mean figures shown in table 6.

TABLE 6. EXPERIMENT WITH SOY BEANS, 1939

Growth of soy-bean nodule bacteria, strain 507. Bacterial cells per ml. in media containing soy-root juices. Means of four replicate cultures

Medium containing root juice from plants		
Uninoculated (control juice)	Inoculated with strains	
	501	507
471	1980	1127
s.e. of difference between means = ± 129.8 , $n = 9$		

The juice from roots bearing the inefficient 507 nodules gave significantly poorer growth of the test organism than did the juice from roots with the efficient 501 nodules, but the control juice behaved in an unusual manner and gave much poorer growth than either of the other juices. It is possible that this unusual result was due to the omission of yeast from the basal

medium. This experiment thus provides the only case in which juice from plants bearing nodules of an inefficient strain gave better growth than juice from nodule-free roots.

DISCUSSION

The composition of root juice is clearly liable to be affected both by growing conditions of the plant and by details in the method of extraction and filtration that are very difficult to control. It is to be expected, therefore, that somewhat variable results will be obtained in experiments of the type described above. The experiments must be considered together in order to assess the validity of conclusions derived from them. They comprise six experiments, three with peas and three with soy beans. The fact that the technique of testing the effect of the juices was varied from one experiment to another will strengthen the conclusions common to all the experiments. In most of the experiments several test organisms were used, and in all of them several types of root juices were compared.

It was pointed out in the introduction (p. 477) that the maladaptation to their host plant shown by ineffective strains might be due to some factor normally present in the root system, that acted specifically against such strains. The growth of the different bacteria in root juice from uninoculated plants, shown in tables 1, 2, 4 and 5, is not correlated with the effectiveness or otherwise of the strain used as test organism. This shows that the root juice does not normally contain any factor that acts specifically against ineffective strains.

On the other hand, there is evidence that the type of nodule on the root system affects the ability of the juice from roots and nodules to support growth of the test organisms. Analyses of variance were made for each experiment, and the standard errors of differences between means obtained. The latter are given for each experiment. From them has been calculated the significance of differences in growth of the nodule bacteria on juices from plants bearing effective and ineffective nodules, and on juice from nodule-free roots. These comparisons have been summarized in table 7, in which a 'plus' sign means that there has been significantly better, and a 'minus' sign significantly poorer growth, while '0' means that the difference did not attain significance. The third column shows the comparative growth of test organisms on juices from roots bearing effective nodules as against growth on juices from roots with ineffective nodules. Out of the forty-four possible comparisons, there were twenty-seven in which the former juice gave significantly the better growth. In all remaining seventeen cases the differences were insignificant.

This effect of root juice upon bacterial growth affords a simple explanation of the observed appearances in effective and ineffective nodules. The rapid growth and long duration of activity amongst the bacteria in effective nodules and the slower growth and early onset of disintegration of the bacteria in ineffective nodules is just the result that might be expected from the different environment provided by the root juice in each case.

The difference in effect of juice from roots bearing effective and ineffective nodules might itself be due to a stimulating action upon bacterial growth of the former type of juice, or to a depressing action of the latter type. These two effects can, to some extent, be separated by comparison with the effects of control juice from nodule-free roots.

Twenty-five comparisons have been made between the effects of juices from roots bearing effective nodules and those of control juice (column 4 of table 7). The former gave significantly better growth of bacteria than control juice in ten cases and significantly poorer growth in three cases.

Twenty-five comparisons were made between growth on juices from plants with ineffective nodules and on control juice (column 5, table 7). In eleven of these the juice from roots with ineffective nodules gave growth significantly poorer than control juice, and in only one case was this result reversed.

The evidence taken as a whole thus indicates that there is an increased stimulating effect from juices of roots bearing effective nodules, possibly connected with the products of nitrogen fixation, while the presence of ineffective strains in the roots induces the appearance of some factor in the root juice which is depressing in its action on bacterial growth.

With regard to the nature of this depressing factor, it should be noted that the test organisms made growth on all the juices that were tested except on two in the first experiment. In no instance could a transmissible lytic agent be found in the juice. This provides strong evidence against the hypothesis that any of the strains tested owe their ineffectiveness to the presence of bacteriophage. Had bacteriophage been present in a concentration sufficient to produce the symptoms observed in the ineffective nodules, the technique of growing the same strain in the presence of filtrates of the crushed nodules and roots should have revealed its presence. The same technique of juice extraction has, in fact, been successfully used to demonstrate the presence of bacteriophage in nodules of peas grown in garden soil.

The present results provide no evidence as to whether the factors affecting the growth of nodules and of their contained bacteria are localized in those nodules or distributed throughout the root system. But it seems probable

that they are localized in or near the nodules; for, *when nodules of both effective and ineffective types develop on the same root system, the characteristic size and appearance of each type of nodule is not affected by the presence of the other type on the same root.*

TABLE 7. SUMMARY OF SIGNIFICANT DIFFERENCES

Experiment	Test organism	3	4	5
		Comparative growth of bacteria on root juices from plants inoculated with		
		Effective strain— Ineffective strain	Effective strain— uninoculated	Ineffective strain— uninoculated
Soy 1936 (table 1)	501	+++	+0	--
	505	+00	+0	00
	502	++	+	00
	507	+00	00	00
Pea 1936 (table 2)	310	+++	++	-0
	317	+000	-0	--
	313	+00	+0	00
	B 33	0+00	00	00
Pea 1937 (table 3)	B 33	++	++	-
Soy 1937 (table 4)	501	+++	00	--
	502	++ +0	+0	-0
	507	0	-	-
Pea 1939 (table 5)	317	0	0	0
	B 33	0	-	-
Soy 1939 (table 6)	507	0	+	+
Totals:				
Significantly greater		27	10	1
Significantly less		0	3	11
Insignificant		17	12	13
Total comparisons		44	25	25

A plus sign means that the first-named root juice produced significantly better, and a minus sign significantly poorer growth than the second-named juice. '0' indicates an insignificant difference.

The evidence as a whole suggests that a soluble substance is formed within the nodules produced by an ineffective strain, that is harmful to the growth of the bacteria. The formation of such a substance would account for the early arrest in nodule growth and for the rapid onset of disintegration of the contained bacteria.

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Competition between related strains of nodule bacteria and its influence on infection of the legume host

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The work deals with the behaviour of mixed strains of nodule bacteria towards each other and towards their legume host.

It introduces the concept of dominance in competition between strains. This dominance is independent of degree of effectiveness as regards nitrogen fixation.

Where two strains of nodule bacteria are both present in the surroundings of their host's root system, active competition between them may cause the strain having the higher initial growth rate almost completely to check multiplication of the other strain outside the plant. This dominant strain will then be responsible for nearly all the nodules.

In peas and soy beans, where growth of the root system is rapid and of comparatively short duration, the nodule-producing capacity of the plant may be partially or wholly satisfied by the nodules produced within the first few weeks, so that further infection, whether by the same or by a different strain, is checked or inhibited.

In clover, whose root system continues to grow over a long period, the first-formed nodules do not stop further nodules from being formed either by the same or by a different strain.

There are large differences in the rates of appearance and final numbers of nodules produced by different strains supplied in pure culture, particularly with clover.

The relative numbers of nodules produced by the two strains simultaneously applied to the roots is conditioned by the specific infectivity peculiar to each strain, unless some other factor, such as competition outside the plant, masks this effect.

1. INTRODUCTION

The factors that control the infection of crop plants by various micro-organisms have been extensively investigated in the hope of being able to check infection by pathogens and of stimulating it in the case of symbiotic organisms. Most of these studies have been carried out with pure cultures, although in a state of nature the micro-organism attacks its host plant in the presence of, and often in competition with, other organisms which may belong to quite different groups or to strains closely related to itself. The

competition between different genera and species of pathogenic and saprophytic fungi in the soil has formed the subject of fruitful study. For references, see a review by Garrett (1939).

Very little is known, however, about the intra-specific competition for infection that may take place when two or more related strains of a pathogenic or symbiotic organism are present, either simultaneously or in succession, in the surroundings of their host plant. This type of competition, when it takes place between strains differing markedly in their effect on the host, must greatly influence the results of infection, and presents a problem of evident importance both to plant pathology and to the practical application of our knowledge of the symbiotic nodule bacteria.

It is a complex problem involving factors such as competition outside the host plant, as well as the relative infective virulence of the strain and the question of a possible acquired immunity developed by the host plant: factors which may operate independently or may interact.

The genus *Rhizobium* offers unique material for the investigation of competitive infection because infection is localized in the form of nodules, a count of which provides a simple measure of infectivity; because it is often relatively easy to identify the strain that has produced a given nodule, and because the host is not killed by the infection which can hence be studied over the full period of the plant's growth.

A field of research of considerable agricultural importance has been opened up by the discovery that certain strains of *Rhizobium* produce nodules that are almost wholly ineffective in benefiting their legume host plant. In particular, this discovery suggests a new application for the process of legume seed 'inoculation' with cultures of nodule bacteria. Hitherto this process has been mainly successful where a leguminous crop, often of comparatively recent introduction, was being grown in a soil otherwise lacking or deficient in bacteria capable of producing nodules upon its roots. However, soils are now known where a legume crop finds a predominant strain of *Rhizobium* which will produce nodules upon it but will not materially benefit its growth. In such soils it should be possible to use the inoculation process to supply the legume crop with an effective strain to replace the ineffective one that it will otherwise acquire from the soil.

But if this replacement is to be a success, the introduced beneficial strain must be capable of competing for nodule formation with the ineffective strain already present, probably in far greater numbers, in the soil. Our information about strain 'effectiveness' will be of little use in extending the benefits of the inoculation technique to soil infested with ineffective

strains until we have some understanding of the factors that control competition for nodule formation when two or more strains, both capable of forming nodules, are present in the root surroundings of the host plant.

The important effects of this competition upon the yield and nitrogen content of the host crop plant are well shown by some work carried out at Rothamsted with the ineffective 'Coryn' strain which infects clover. This strain was isolated from stunted clover plants which characterized the pastures on Coryn Mountain in central Wales. Preliminary experiments with clover grown in pots of sand indicated that when this strain was present in the sand it reduced the growth of the plant even when effective strains were also present (see experiment 1). A search was therefore made for an effective strain capable of producing a good growth of clover in sand containing Coryn strain bacteria. A strain satisfactory in this respect was obtained from Professor Ch. Barthel, Central Agricultural Experiment Station, Stockholm, for whose kind assistance thanks are due. This strain is referred to below as strain A.

The following experiment illustrates the effect of the Coryn strain on the growth of clover in the presence of strain A and of a mixture of strains of clover *Rhizobium*, effective by themselves but liable to be suppressed when in competition with the Coryn strain.

Experiment 1

Montgomery red clover was grown in glazed earthenware pots each containing 12 kg. of nitrogen-deficient sand. One litre of the following nutrient solution was added:

K_2SO_4	0.9 g.	$MgSO_4 \cdot 7H_2O$	0.5 g.
$CaSO_4 \cdot 2H_2O$	0.3 g.	NaCl	0.5 g.
K_2HPO_4	0.3 g.	$FeCl_3$	0.04 g.
KH_2PO_4	0.3 g.	Tap water	1 l.

All the pots received a mixed inoculum of efficient strains of clover nodule bacteria derived from Rothamsted soil.

The sand was not sterilized because it was hoped that wild strains of bacteria casually introduced might contain amongst them some that were capable of competing effectively with the Coryn strain. This hope was not realized, but later, a strain having this desirable character was isolated from Agdell Field, Rothamsted. This strain (labelled W) was included in experiment 2.

Set 1 received only the wild local strains, set 2 received in addition a suspension of the Coryn strain, set 3 of strain A, and set 4 of both these strains in approximately equal numbers. These inocula were added to the food solution before it was added to the sand. Set 1 consisted of five and the remaining sets of fifteen replicate pots; each pot contained five plants. The seed was sown on 11 April. The resulting dry weights and nitrogen contents of the plants obtained after 14 weeks' growth, are shown in table 1.

TABLE 1. EXPERIMENT 1. RED CLOVER GROWN IN
NITROGEN-DEFICIENT SAND

set	no. of pots	strains of <i>Rhizobium</i> supplied	mean dry weights per pot g.	standard errors	N content of plants, means per pot mg.	standard errors
1	5	mixed Rothamsted strains only	28.00	± 1.32	701	± 34.4
2	15	mixed Rothamsted strains plus Coryn strain	8.95	± 1.91	218	± 46.3
3	15	mixed Rothamsted strains plus strain A	26.84	± 0.85	673	± 32.7
4	15	mixed Rothamsted strains plus Coryn strain and strain A	21.89	± 1.57	550	± 40.7

The mixed local strains without further addition (set 1) produced a good growth of clover with a mean dry weight per pot of 28 g. containing 701 mg. of nitrogen. The addition of the Coryn strain to these mixed strains in set 2 reduced their effect so that the clover in set 2 gave a mean yield of 8.95 g. containing 218 mg. of nitrogen. But in the presence of strain A, the Coryn strain reduced the growth and nitrogen fixation to a very small though significant extent (set 4).

Experiment 2

The effects on the growth of red clover of the effective strains A, W and the Wisconsin strain 205 alone and in combination with the ineffective strains Coryn and Wisconsin strain 202 were tested in a pot experiment in nitrogen-deficient sand. The two Wisconsin strains used were amongst those employed by Dunham and Baldwin in their work on double inoculation (1931). The clover was grown in glazed earthenware pots containing 12 kg. of sand supplied with the same nutrient solution as was used in experiment 1.

Sets of pots were supplied with each of the effective strains alone and in combination with each of the ineffective strains. Suspensions of the bacteria were added to the nutrient solution before pouring on to the sand. In the sets receiving two strains, approximately equal numbers of cells of each strain were mixed in suspension before addition to the sand. The sand was not sterilized because the previous experiment had shown that it did not contain strains capable of competing with the Coryn strain. Montgomery red clover was sown on 23 March and grown for 14 weeks. The dry weights and nitrogen contents then found are shown in table 2.

TABLE 2. EXPERIMENT 2. RED CLOVER GROWN IN
NITROGEN-DEFICIENT SAND

set	no. of pots	strains of <i>Rhizobium</i> supplied	mean dry weights per pot g.	standard errors	N content of plants, means per pot mg.	standard errors
1	10	A	9.13	± 0.91	230	± 27.3
2	10	W	8.90	± 0.40	228	± 13.7
3	10	205	7.23	± 0.46	214	± 10.7
4	10	A + C	9.54	± 0.60	257	± 18.7
5	10	W + C	9.87	± 0.97	260	± 21.9
6	15	205 + C	0.29	± 0.23	4	± 1.0
7	10	A + 202	11.31	± 0.52	280	± 19.9
8	10	W + 202	9.85	± 0.75	244	± 20.5
9	10	205 + 202	5.08	± 0.62	162	± 13.9

The three effective strains produced a good growth of clover in the absence of the ineffective strains (sets 1-3). The normally effective strain 205 was almost incapable of supporting growth of the clover in the presence of the Coryn strain (set 6). The effect of strain 205 was also significantly reduced by the presence of the ineffective strain 202 (set 9). But the effects of the dominant and effective strains A and W were not significantly affected by the presence of the large inocula of Coryn or 202 added to the sand in sets 4, 5, 7, and 8. The results of these two experiments are most easily explained on the assumption that certain strains prevent or check nodule formation by other strains that are simultaneously present in the root surroundings. This check might be due either to competition between the strains outside the plant or to nodules first produced by one strain conferring an immunity in the plant against the entry of the other strain. Israily (1929) claimed that such an immunity could be induced.

Marie Löhnis (1930) made experiments with red clover inoculated with Wisconsin strains 205 and 202. She found that when the plants were supplied with one of these strains at sowing time, re-inoculation with the same or with the other strain after about a month's growth caused no significant increase in nodule numbers. She grew her plants in tubes of agar medium, under which conditions poor growth of the plant usually limits nodule numbers to a low figure which might well have been reached in month-old plants.

Dunham and Baldwin (1931) made experiments with lucerne, clover, peas and soy beans, using, with each host plant, an effective and an ineffective strain of nodule organism, the two strains being applied to the same plants, in some sets simultaneously at sowing time, and in other sets in succession, one strain at sowing time and the other after an interval of 4-6 weeks. After simultaneous inoculation, the two strains both usually produced nodules. With successive inoculation, in six sets out of eight the first-applied strain produced all the nodules that were tested, the remaining two sets giving nodules by both strains. In a second experiment using clover inoculated at sowing with strain 202 and after 56 days re-inoculated with strain 205, the first strain contributed all of the 100 nodules tested.

This preponderating effect of the first-applied strain could, on the evidence, be explained by several hypotheses:

(a) The growth of the plant may have been slowed down to such an extent by the time the second inoculation was made that nodule formation by either strain had ceased.

(b) The first strain may have continued to produce nodules after the second inoculation but it may have immunized the plant specifically against the second strain. This explanation is the one favoured by the authors.

(c) The nodule-bearing capacity of the plant may have been satisfied by the first-applied strain so that, by the time of the second inoculation, few or no further nodules could be produced by either strain. Neither this nor explanation (a) is sufficient to explain the results of Dunham and Baldwin's second experiment with clover, where they refer to the formation of nodules on new roots. But either explanation might apply to their results with other plants.

(d) The first strain may have swamped the second strain and prevented its multiplication *outside* the plant. In this work the second strain was poured on to the sand which already contained a presumably large population of the first strain.

2. FACTORS AFFECTING THE COMPETITION FOR NODULE PRODUCTION BETWEEN STRAINS OF PEA AND SOY BEAN *RHIZOBIUM*

Experiment 3

The following experiment was made in the hope of confirming some of Dunham and Baldwin's results and of testing the applicability of some of the explanations given above. Dwarf peas were grown in glazed earthenware pots each containing 3 kg. of nitrogen-deficient silver sand and supplied with the same nutrient solution as was used in the first two experiments. The sand was rendered free from nodule bacteria by blowing steam for 30 min. through a hole in the base of the pot. The nutrient solution was sterilized and watering was carried out with boiled rain water.

Two strains of nodule bacteria were used in the experiment, the effective strain Wisconsin 310, used by Dunham and Baldwin in their experiment, and the ineffective strain B 33, also obtained from Wisconsin.*

Set 1 received an inoculum of strain B 33 at the time of sowing, and was given a further heavy dose of the same strain when the plants were 6 weeks old. In set 3 the same strain was applied only after the peas had grown for 6 weeks without nodule bacteria.

Sets 4 and 6 were similarly inoculated with strain 310: set 4 receiving the bacteria both at sowing time and after 6 weeks, set 6 only after 6 weeks.

Set 2 received strain B 33 at sowing time and strain 310 after 6 weeks. Set 5 received strain 310 at sowing time and strain B 33 after 6 weeks.

Sets 7 and 8 received an inoculation of both strains applied simultaneously, suspensions of each strain containing approximately equal numbers of bacteria being mixed before application to the sand. In set 7 this mixture was applied both at sowing time and after 6 weeks and in set 8 only after 6 weeks.

An uninoculated control set was also included. The peas in this set remained free from nodules.

The inocula were applied in the food solution. The pots were allowed to dry somewhat before adding the second inoculation, to ensure that it mixed well with the sand. Each set consisted of four replicate pots except sets 1 and 4 which consisted of six pots each, from two of which the plants were removed after 6 weeks and their nodules counted. Four peas were grown in each pot from externally sterilized seed. After 16 weeks' growth, the remaining plants were removed from the pots and their nodules counted.

* The authors' thanks are due to the Staff of the Wisconsin Agricultural Experiment Station for their kindness in supplying cultures of the strains of pea and soy bean *Rhizobium* used in this work and also of strains 202 and 205, isolated from clover.

Cultures were isolated from sixty nodules, fifteen taken at random from each pot, in each set that had received both strains. The cultures thus isolated were identified as belonging to strain 310 or B 33 according to the appearance of their growth on yeast agar slopes, which is very distinct as between the two strains. From these identifications the percentage of nodules belonging to each strain were estimated.

TABLE 3. EXPERIMENT 3. PEAS GROWN IN SAND

set	strains supplied		mean number of nodules per pot				percentage containing	
	at sowing	after 6 weeks	after 6 weeks	standard errors	after 16 weeks	standard errors	strain B 33	strain 310
1	B 33	B 33	550	± 32.1	548	± 25.0	—	—
2	B 33	310	—	—	473	± 25.8	96.1	3.9
3	—	B 33	—	—	266	± 43.0	—	—
4	310	310	332	± 51.5	417	± 42.0	—	—
5	310	B 33	—	—	417	± 9.8	11.6	88.4
6	—	310	—	—	306	± 39.0	—	—
7	B 33 + 310	B 33 + 310	—	—	434	± 19.0	95.5	4.5
8	—	B 33 + 310	—	—	285	± 5.5	94.6	5.4

The results of the experiment are set out in table 3. Sets 3 and 6, which were not inoculated until the plants were 6 weeks old, subsequently developed a mean of 266 and 306 nodules per pot respectively, thus showing that peas of this age were not too old to develop plenty of nodules.

Nodules produced during the first 6 weeks reduced the subsequent nodule formation from a re-inoculation made at this time. But this inhibition affected further nodule formation by the same strain as much as by a different strain. Thus strain B 33 produced 550 nodules per pot in the first 6 weeks (set 1) and the numbers found after, at 16th week, were not increased either by re-inoculation with the same strain (set 1) or by the other strain (set 2), though in the latter set a few nodules were produced by the second applied strain. Strain 310 produced 332 nodules per pot in the first 6 weeks. Re-inoculation with the same strain increased the mean number of nodules per pot by a further 85, developed between the 6th and the 16th week, resulting in a total of 417 nodules (set 4). But plants that bore no nodules during the first 6 weeks and were then supplied with strain 310, developed 306 nodules by the 16th week (set 6). Hence the presence of nodules during the first 6 weeks reduced the number of nodules subsequently produced from 306 to 85. The nodules formed during the first 6 weeks by strain 310 had a similar effect in reducing subsequent nodule formation in plants re-inoculated with strain B 33 (set 5). The root system

of the plant thus has only a limited capacity for producing nodules: this limit was reached within the first 6 weeks by plants supplied with strain B 33 but was not attained during this initial period by plants supplied with strain 310 (sets 1 and 4).

The final nodules produced in set 5 show an increase of 85 on the 332 nodules produced during the first 6 weeks by strain 310. This figure represents about 20% of the total 417 nodules produced during the whole growth period. Since 11.6% of this total consisted of B 33 nodules, about half the nodules produced after the application of strain B 33 were due to this strain. Thus the experiment provides no evidence that the first-applied strain confers any specific immunity against subsequent nodule formation by a different strain. For, when strain B 33 was first applied (sets 1 and 2), the nodules formed during the first 6 weeks prevented any further increase in nodule numbers by either strain, while the lesser number of nodules produced by strain 310 within the first 6 weeks allowed an equal chance for further nodules to be produced by bacteria of strain 310, still present in the sand, or by those of strain B 33, subsequently added (set 5).

When the two strains were applied simultaneously in about equal numbers (sets 7 and 8) the ineffective strain B 33 dominated the nodule formation. This dominant strain also differed from strain 310 in that, when applied alone at sowing time, it enabled the plant to reach the limit of its nodule-producing power within the first 6 weeks (compare sets 1 and 4).

Experiment 4

Another pot experiment with peas was made to test the dominance of one strain over another when both were simultaneously applied. In this experiment, one set of four replicate pots was supplied with a mixed culture in about equal numbers of strains 310 and B 33, and another set with a mixture of strains 310 and 313, the latter being the ineffective strain which, together with strain 310, was employed in Dunham and Baldwin's work. The technique of this experiment was similar to the one above described. The plants were grown from 8 March till 12 June, when the nodules were counted and isolations of the bacteria were made from forty nodules of each set (ten per pot). The set given a mixed inoculum of strain 310 and B 33 produced a mean of 685 nodules per pot, 93% of those tested containing strain B 33. The set given the mixture of strains 310 and 313 produced 703 nodules, strain 313 contributing 90% of those tested. In these experiments with peas, two strains, both ineffective, in nitrogen fixation, were strikingly dominant as regards nodule production over the effective strain 310, when applied at the same time as the latter.

Experiment 5

An experiment on lines similar to experiment 3 was carried out with soy beans, which were grown in glazed earthenware pots each containing 12 kg. of nitrogen-deficient sand and supplied with 1 l. per pot of the same nutrient solution used in the previous experiments. The sand and nutrient solution were not sterilized, as it had been found that bacteria capable of producing nodules on soy beans were not naturally present in either. Two strains of nodule bacteria, both obtained from Wisconsin Agricultural Experiment Station, were used in this work; strain 501—an effective strain also used by Dunham and Baldwin (1931)—and the ineffective strain 507. The plant of the experiment was similar to that of experiment 3 except that the re-inoculations were carried out after 9 weeks' growth and that four instead of two additional pots inoculated at sowing time with each strain, were set up for the purpose of counting the nodules found at the time of the second inoculation. The remaining pots were kept on until the 16th week, when the nodules were counted and examined.

The nodules produced by the two bacterial strains 501 and 507 possess the fortunate character of being readily distinguishable from free-hand sections. Those produced by the effective strain 501 are soft in the centre, which is either dark red or olive green in colour; those produced by the ineffective strain 507 are hard in the centre, which is either white or very pale clear green. These differences are due to the different proportions of cells containing bacteria and of sterile starch-filled cells in the central tissue of the nodule (see Chen and Thornton 1940). Two hundred nodules produced by each strain in pure culture were cut across and every nodule could be correctly ascribed by this test to the strain which produced it.

At the conclusion of the experiment nodules were counted and about 100 nodules per pot were identified by free-hand sections. From these identifications the percentage of nodules produced by each strain was calculated for each of the sets with mixed inoculations. The results of this experiment are shown in table 4.

In this experiment, as in the last, the nodules produced on the young plants tended to inhibit the further formation of nodules by either strain. Strain 501 produced 247 nodules per pot by the 9th week; and re-inoculation by either strain at this time failed to cause any significant increase in nodule numbers by the 16th week (sets 1 and 2), although plants whose roots were nodule-free up to the 9th week and which were then supplied with strain 501 developed 229 nodules (set 3), and similar plants supplied at this time with strain 507 produced 159 nodules between the 9th and the

16th week (set 6). Strain 507 produced 129 nodules by the 9th week: after re-inoculation with the same strain, plants in set 4 finally produced 191 nodules, an increase of 62 nodules developed between the 9th and the 16th week. The difference between this increase and the 159 nodules produced by this strain during the same period on sterile roots in set 6 is 97 ± 27.18 and is quite significant.

TABLE 4. EXPERIMENT 5. SOY BEANS GROWN IN SAND

set	strains supplied		mean numbers of nodules per pot				percentage containing	
	at sowing	after 9 weeks	after 9 weeks	standard errors	after 16 weeks	standard errors	strain 501	strain 507
1	501	501	247	± 3.59	254	± 5.6	—	—
2	501	507	—	—	239	± 19.3	100	0
3	—	501	—	—	229	± 14.3	—	—
4	507	507	129	± 6.10	191	± 8.5	—	—
5	507	501	—	—	267	± 17.4	27	73
6	—	507	—	—	159	± 25.1	—	—
7	501+507	501+507	—	—	185	± 28.0	98	2
8	—	501+507	—	—	265	± 42.7	98	2

This experiment also failed to produce evidence that any greater immunity was conferred by the early nodules against a different strain subsequently applied, than against a further application of the same strain. In set 5, supplied with strain 507 at sowing time and with strain 501 after 9 weeks, the final nodule numbers, averaging 267 per pot, show an increase of 138 on the 129 nodules produced by strain 507 up to the time when strain 501 was applied. Thus, about 52% of the nodules found at the end of the experiment were produced after the second inoculation. Since 27% of the total 267 nodules were of the 501 type, this strain must have contributed about half of the nodules produced since it was applied. So that in set 2, where strain 501 was first applied, the nodules produced in the first 9 weeks prevented further nodule production by either strain, while in set 5, where strain 507, applied at sowing time, did not reach the limit of nodule production by the 9th week, the addition of strain 501 to the sand which still contained the first-applied strain 507, allowed each strain an equal chance to produce nodules.

When the two strains were applied simultaneously in about equal numbers (sets 7 and 8), one strain—in this case the effective strain 501—was dominant and produced 98% of the nodules. The dominant strain again

differed from the other in that, when applied alone at sowing time, it satisfied the nodule-producing power of the plant by the time of the second application of bacteria.

The conclusions derivable from these last two experiments are thus very consistent. In both of them one strain dominated the other when both were simultaneously applied. In both, the dominant strain, when applied at sowing time, had almost or quite satisfied the nodule-producing capacity of the plant before the time of the second application of culture, so that neither strain could produce further nodules. But when the dominant strain was applied second, to plants the limit of whose nodule-producing power was not reached, both strains had an equal opportunity to produce the remaining nodules.

The above experiments enable us to eliminate some of the possible explanations of Dunham and Baldwin's results, that were suggested and lettered above (p. 37):

(a) In our experiments, peas and soy beans first inoculated after 6 and 9 weeks respectively, produced plenty of nodules. The failure of the second-applied strain to produce nodules thus cannot be due merely to the age of the plant, either in our experiments or, by inference, in the work of Dunham and Baldwin who applied the second strain to plants of about the same age.

(b) In our experiments the first-applied strain did not immunize the plant specifically against a different strain.

(c) The nodules developed on the young plant tended to inhibit further nodule formation, either by the same or by a different strain, because their numbers approached or even reached the limit set by the nodule-bearing capacity of the root system. This limit was sometimes reached by the time the second culture was applied and may thus explain the failure of Dunham and Baldwin's peas and soy beans to develop nodules from the second-applied strain.

(d) When two strains are applied simultaneously in equal numbers to the surroundings of the root, it seems most unlikely that the almost complete dominance of one of them, as found in experiments 3, 4 and 5, could have been due to the dominant strain saturating the root system with nodules before the other strain was able to infect the roots, unless competition between the strains outside the plant enabled the dominant strain initially to repress the other in the root surroundings.

The following experiment was made in order to search for such a competition in the sand surrounding the root system.

Experiment 6

For this experiment dwarf peas were used together with the effective strain 310 and the ineffective strain 313. Experiment 4 had shown that the latter strain was strongly dominant as regards nodule formation when both strains were simultaneously supplied to peas grown in sand. In the present experiment, dwarf peas were grown in quart milk bottles, each containing 800 g. of nitrogen-deficient sand mixed with 1 g. of precipitated chalk. The bottles of sand were stoppered with cotton-wool and sterilized in the autoclave for 1 hr. at 15 lb. pressure. 100 ml. of the same food solution as was employed in the previous experiments were added to each bottle. Twelve replicate bottles were supplied with a pure suspension of strain 310 and twelve with a pure suspension of strain 313, each strain at a concentration giving about 1,000,000 bacterial cells per gram of sand. Twenty-four bottles were supplied with a mixed suspension giving about 1,000,000 bacteria of each of the two strains per g. of sand.

The food solution was autoclaved and the appropriate bacterial suspension mixed with it before adding it to the bottles.

All these bottles were sown with peas at the rate of six per bottle. The seed was sterilized externally by immersion in absolute alcohol for 3 min. and in a 0.2% aqueous solution of HgCl_2 for a further 3 min. and washed in four changes of sterile water.

In addition to the above sets eight bottles were given a mixed inoculum of the two strains as already described but were not sown with peas.

The experiment was set up on 28 July and plate counts of the bacteria in the sand were made from the bottles sown with peas at the beginning and after 7, 16, 22, 29 and 60 days, and from the bottles without peas after 7, 16, 22 and 60 days. On each occasion duplicate bottles were sampled from the sets given pure cultures and from those without peas, and four replicate bottles from the set given a mixed inoculum and sown with peas. About 50 g. of sand were removed from each bottle into a sterile petri dish and well mixed with a flamed spatula. From this platings at several dilutions were made on yeast agar.*

The surface colonies of strains 310 and 313 are distinguishable, the former producing opaque white and the latter thin watery colonies. It was there-

* The medium used for plate counts had the following composition:

K_2HPO_4	0.5 g.	Yeast water (15% yeast)	100 ml.
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.2 g.	Tap water	900 ml.
NaCl	0.2 g.		
CaCO_3	3.0 g.		

fore possible to make separate counts of the surface colonies of each strain in platings of a mixed culture. In order to make this separation possible, counts of colonies in all sets were limited to surface colonies. The bacterial numbers calculated were therefore too low but relatively correct.

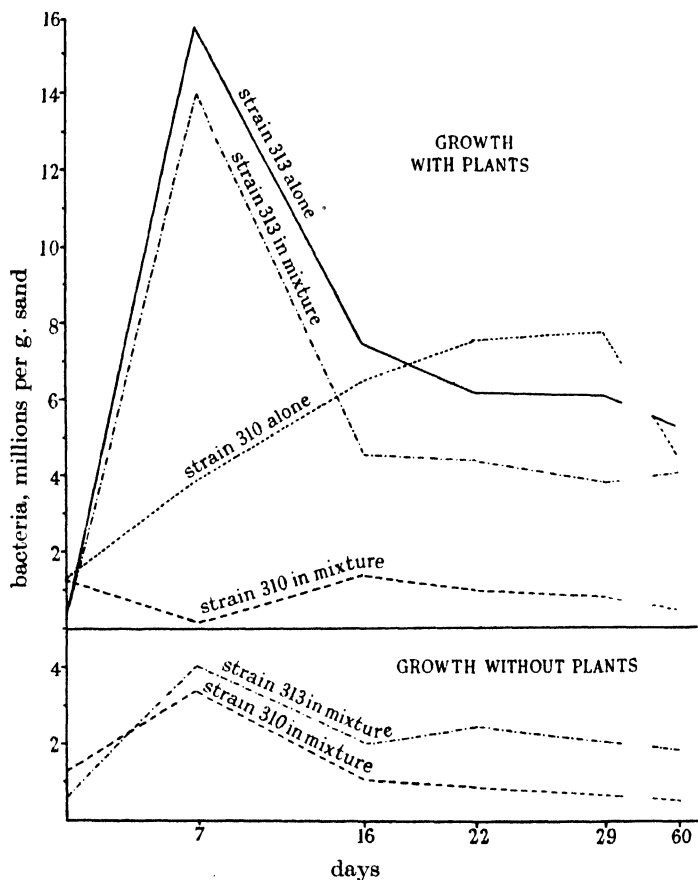


FIGURE 1. Growth of pea nodule bacteria in sand with and without plants (experiment 6).

The results of this experiment are graphically shown in figure 1, in which counts from each strain growing alone and in mixture with the other, are separately plotted. In the sets containing growing peas, counts made after 7 days showed a large rise in the numbers of strain 313 both in pure and in mixed culture. The numbers had fallen by the 16th day after which they remained fairly steady. The presence of strain 310 slightly decreased the numbers of strain 313 as compared with those reached by the latter in

pure culture. In the presence of the plants, strain 310 in pure culture grew at first much more slowly but showed a steady increase up to the 29th day. But in mixed culture the increase of strain 310 was entirely prevented by competition with the faster-growing dominant strain 313. The percentage of the mixture composed of strain 313 varied at different times, but the mean percentage over the run of the experiment was 88.4. The proportion of the two strains in the sand in the presence of the plant is therefore in good agreement with that of the nodules developed by each from a mixture of these two strains applied to the plant in experiment 4, where 90 % of the nodules contained strain 313. This suggests that the two strains, when in contact with the root, have an equal chance of infecting the plant, the relative number of nodules developed by each strain being an expression of the proportions of the two strains in the sand surrounding the roots, a proportion resulting from competition between them.

The complete suppression of growth of strain 310 by the dominant strain 313 would seem to be related to the early rapid growth of the latter strain. Hence when a normally dominant strain is applied some weeks after the other strain to root surroundings already populated by the latter, the dominance should not show itself. This conclusion was borne out by experiments 3 and 5. In set 5 of experiment 3 strain 310 was applied at sowing time and strain B 33 after 6 weeks. The two strains contributed about equally in producing the nodules that subsequently developed; although strain B 33 is strikingly dominant when applied simultaneously with strain 310 (sets 7 and 8, experiment 3). A similar phenomenon appears in the soy bean (experiment 5).

The early rapid growth of the dominant strain also offers an explanation for the early saturation of the nodule-producing power of the plant by dominant strains when applied at sowing time; this holds for both peas (experiment 3) and soy beans (experiment 5).

In the set without peas in the present experiment the number of bacteria of either strain did not rise above the 4 millions per g. of sand at any time of sampling and there was in consequence no evidence of any competition between the strains. The rapid increase in strain 313, and its inhibiting effect on strain 310, are thus related to the presence of the plant's roots, although taking place outside them. The experiment indicates that the roots secrete an energy source or an accessory growth substance that stimulates the growth of both strains but especially that of strain 313. An experiment was therefore made to test the effect of replacing the root secretions by a pure carbohydrate as energy source.

Experiment 7

The same method was, in general, followed in this as in the last experiment, save that no peas were grown in any set. Twenty-eight bottles received the basal food solution without carbohydrate and twenty-eight a further addition of 0.2% sucrose. Eight bottles of each medium were given a suspension of strain 310, eight bottles received a suspension of strain 313 and the remaining twelve bottles a mixture of the two strains. The cultures were applied at a concentration giving about one million bacteria of each strain per g. of sand. Plate counts of the two strains were made at the start and after 4, 6 and 13 days using the method described for experiment 6. Counts of the pure cultures were made on each occasion from duplicate bottles and of the mixed cultures from triplicate bottles. The results are shown in figure 2.

In the bottles without either plants or added energy supply, the number of cells of either strain, alone or in mixture, did not rise above 5.1 millions per g. and there was no evidence of competition between the two strains. This is in agreement with the behaviour of the similar sets in the last experiment. In the presence of sugar, strain 313 when in pure culture rose to 28 millions in 4 days, after which its numbers fell to 17.3 millions by the 13th day. In the presence of the other strain the numbers of strain 313 rose to 19.1 millions by the 4th day. Strain 310 when in pure culture grew much more slowly, reaching only 10.1 millions by the 4th day, and thereafter rising steadily to 27.5 millions by the 13th day. In mixture with the dominant strain 313, the growth of strain 310 was again severely checked, the numbers of this strain never rising above 3.6 millions.

The course of growth of the two strains in this set with added dextrose closely resembles that found in the last experiment when pea roots were growing in the sand. We have in both cases the same rapid rise in numbers of the dominant strain whether grown alone or in mixture, and the slower growth of strain 310, which is almost inhibited by the presence of strain 313.

The experiments described above suggest the following explanation of the behaviour of two strains, one dominant over the other, as exemplified by the pea nodule strains here studied.

The plant's root system secretes substances that stimulate the multiplication of nodule bacteria in the root surroundings, but the rate of multiplication thus induced differs greatly with different strains. A strain which at first multiplies rapidly under these conditions will quickly satisfy the nodule-producing power of the plant and prevent the

further nodules being formed either by itself or by any other strain supplied later.

When a strain that multiplies more slowly at first is applied in pure culture at sowing time, it will take longer to reach the limit of nodule

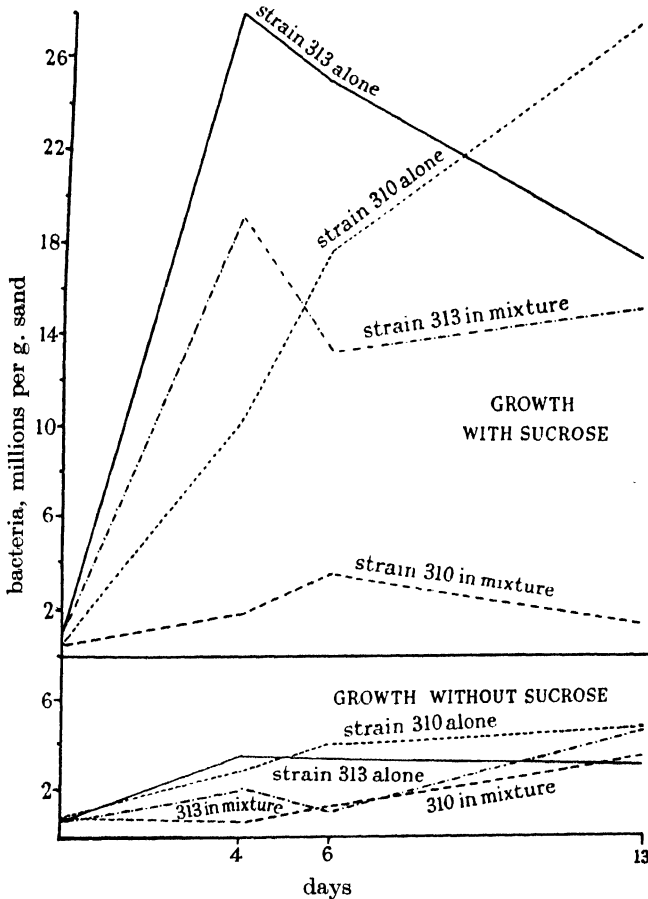


FIGURE 2. Growth of pea nodule bacteria in sand without plants (experiment 7).

production, so that further nodules may be produced by a different strain later applied.

When two strains with different multiplication rates are supplied together, the strain that multiplies more rapidly at first will compete with, and suppress the growth of, the other strain, and will largely prevent it from forming nodules.

3. FACTORS AFFECTING THE COMPETITION FOR NODULE PRODUCTION BETWEEN STRAINS OF CLOVER *RHIZOBIUM*

In the experiments with peas and soy beans, a limit to the nodule-bearing power of the root at a fairly young stage of the plant was an important factor, which was sufficient to explain the high percentage of nodules produced by the first-applied strain in sets receiving successive inoculation with two strains, and which must have increased the importance of early competition outside the roots between two strains, both supplied together at sowing time.

This early limitation would seem to be connected with the short life of the pea and soy bean, whose root systems make a large part of their growth within the first 6 weeks. In clover, the root system grows comparatively slowly at first but continues its growth for a long period. In this plant therefore no early limitation to the number of nodules is to be expected.

The following experiment was made in 1939 to determine whether, in the clover plant, the first-formed nodules exerted any inhibiting effect on the further production of nodules by the same or by a different strain.

Experiment 8

Red clover was sown in quart milk bottles each containing sand and 100 ml. of the following food solution:

K_2HPO_4	1.0 g.	$CaCl_2$	0.1 g.
$MgSO_4 \cdot 7H_2O$	0.2 g.	$FeCl_3$	0.02 g.
NaCl	0.1 g.	Water	1 l.

Six replicate bottles were inoculated with strain A, six with the Coryn strain and eight were left uninoculated. The seed was sown on 26 July and on 7 September the plants were removed, their roots washed in sterile water and the nodules counted. The sand from the bottles supplied with the two strains was placed in separate earthenware pans, four pans containing sand infected with each strain. The seedlings were then replanted in these pans so that each pan contained two plants bearing nodules produced by each strain, and two plants without nodules at the time of transplanting. After 2 months' further growth the plants were removed and their roots examined. Some of the plants died, the numbers that survived being shown in table 5. The check due to planting out had produced a local thickening on each root, which made it easy to determine what portion had grown since then. This callus formation enabled a distinction to be drawn between the nodules

formed on the old roots, and those produced on the new roots and hence undoubtedly due to fresh infections from the sand. The results are shown in table 5.

TABLE 5. EXPERIMENT 8. NODULE DEVELOPMENT ON TRANSPLANTED CLOVER

set	no. of plants	original nodules strain	mean no. of nodules when transplanted	standard errors	final nodule numbers	standard errors	increase	no. of nodules on new roots	standard errors
Transplanted into sand containing the Coryn strain									
1	4	A	14	± 2.6	90	± 15.2	76	74	± 16.6
2	9	Coryn	34	± 3.1	118	± 10.1	84	76	± 11.3
3	5	—	—	—	71	± 13.6	71	71	± 13.6
Transplanted into sand containing strain A									
4	10	A	23	± 3.8	55	± 7.0	32	30	± 5.7
5	4	Coryn	32	± 3.7	78	± 12.7	46	43	± 10.4
6	6	—	—	—	46	± 8.9	46	46	± 8.9

The number of nodules produced on the new roots has been quite unaffected by the presence of nodules at the time of transplanting. These first-formed nodules have not checked the further development of nodules either by the same or a different strain. There is, on the other hand, a characteristic difference in the number of nodules produced on the new roots by the two strains. The plants growing in pans containing Coryn bacteria developed a mean of 73.4 nodules per plant on the new roots, those in pans containing strain 205, a mean of only 39.9 nodules.

There are, indeed, great differences in the rates of nodule production on clover by different bacterial strains. Three strains were used in the next part of the work, namely, the effective strains 205 and A and the ineffective Coryn strain. The rates of nodule production on red clover seedlings grown on agar slopes* and supplied with each of these three strains are shown graphically in figure 3. Each strain gives a characteristic curve. Coryn produces a few nodules between the 10th and 20th day and thereafter there is a rapid increase in the number of nodules produced. Strain A begins to produce a few nodules at the same time as Coryn but nodule production thereafter is much slower. Strain 205 does not produce its first nodules until about 10 days after the other two strains have done so, but later the

* The composition of this agar medium was similar to that used in experiment 9 (p. 52).

number of nodules formed by this strain increases at about the same rate as do those of strain A. Since in clover the number of nodules is not determined by the establishment of a limit to further infection in the early stages of the plants' growth, their number will be dependent on the specific infectivity of the strain forming them.* Hence, when equal numbers of two strains are simultaneously applied to the root surroundings, the number of

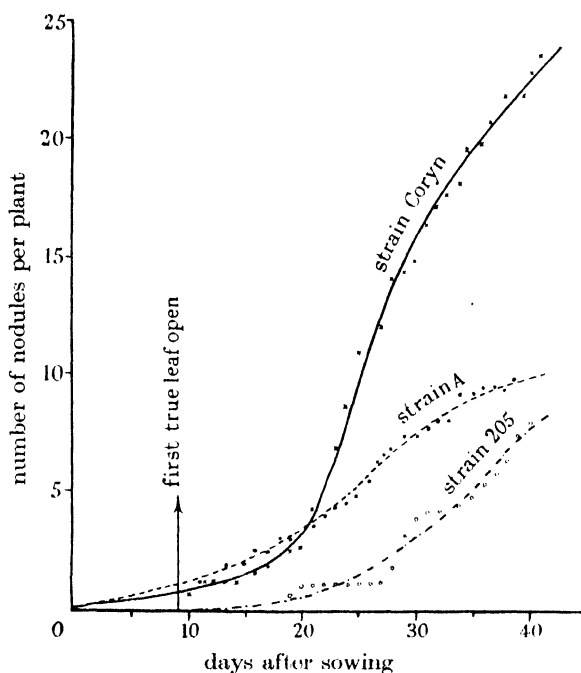


FIGURE 3. Rate of appearance of clover nodules in agar culture.

nodules due to each strain in the mixture should be proportionate to the numbers produced by each strain in pure culture, unless some other factor such as competition outside the root interferes.

The following experiment was made to measure the proportion of nodules produced by each strain when bacteria of two strains were simultaneously applied to the root surroundings in about equal numbers. Two such pairs of strains were tested, namely, Coryn with 205 and Coryn with A. These two pairs were chosen because experiment 2 had shown that the ineffective strain Coryn depressed the growth and nitrogen fixation otherwise produced by strain 205 but was without effect on the action of strain A.

* A further investigation of specific infectivity is described by Chen (1941).

Experiment 9

Red clover was grown in quart milk bottles containing 800 g. of nitrogen-deficient sand and 100 ml. of the following food solution:

K_2SO_4	8.75 g.	$MgSO_4 \cdot 7H_2O$	5.0 g.
$CaSO_4 \cdot 2H_2O$	3.0 g	NaCl	5.0 g.
K_2HPO_4	2.75 g.	$FeCl_3$	0.02 g.
KH_2PO_4	3.0 g.	Water	1 l.

The bottles with sand were sterilized for 1 hr. and the food solution for 15 min. at 15 lb. pressure before addition to the bottles. The bottles were inoculated according to the following plan, the bacterial suspension being added to the food solution in such a manner that all sets were given about equal total numbers of bacteria and those treated with a mixed inoculum received about equal numbers of each strain.

set	inoculation	no. of replicate bottles
1	strain 205	16
2	strain 205 + Coryn	8
3	Coryn	12
4	A	24
5	A + Coryn	12

About fifteen seeds, externally sterilized with absolute alcohol and $HgCl_2$, were sown in each bottle on the surface of the sand. After 3 months' growth the clover roots were washed, the nodules counted and the lengths of a number of nodules, varying from 30 to 100 per bottle according to the strain, were measured. In the sets given double inoculations the percentages of nodules containing each strain were estimated from tests made on cultures isolated from nodules taken at random, and these estimates were checked by the frequency distribution of nodule lengths, as described below. The experiment falls naturally into two parts, that dealing with the interaction of strains Coryn and 205 and that dealing with the interaction of Coryn with strain A. These will be separately considered.

The Coryn strain is difficult to distinguish *in vitro* from strain 205 owing to its close similarity in growth and in physiology. It can, however, be distinguished in the plant by three characters: (a) nodule formation on the seedling grown in agar commences about 10 days later with strain 205 than with the Coryn strain (see figure 3); (b) 205 nodules, when they reach a certain size, are usually of a distinct pink shade; (c) Coryn nodules have a much smaller mean length than those produced by strain 205.

Cultures were isolated from 130 nodules (about 16 per bottle) taken at random from plants of set 2, that were given the mixed inoculum of strains 205 and Coryn. These were supplied to red clover seedlings grown in an agar medium having the following composition:

K_2HPO_4	0.5 g.	$Ca_3(PO_4)_2$	2.0 g.
$MgSO_4 \cdot 7H_2O$	0.2 g.	$FeCl_3$	0.01 g.
NaCl	0.1 g.	Agar	10.0 g.
$FePO_4$	1.0 g.	Water	1 l.

The time of appearance of the first nodule was noted and, after further growth, the presence or absence of pink coloration in the larger nodules was noted. These two tests were in good agreement and indicated that 89.3% of the cultures were of the Coryn strain.

The number of nodules per plant grown in the milk bottles, and their mean lengths, are shown in table 6. The nodule lengths provide a check on the estimate of the composition of the mixed set formed from the

TABLE 6. EXPERIMENT 9. CLOVER GROWN IN QUART BOTTLES.
INTERACTION OF STRAINS 205 AND CORYN

set	no. of plants	strains supplied	mean nodule numbers		percentage of Coryn nodules estimated from isolations		mean nodule lengths in mm.	standard errors	no. of nodules measured
			per plant	standard errors					
1	184	205	16	± 3.4	—		1.13	± 0.025	534
2	115	205 + Coryn	56	± 5.3	89.3		0.53	± 0.01	564
3	146	Coryn	64	± 0.8	—		0.46	± 0.02	840

isolations. The mean length of Coryn nodules on plants of set 3 supplied with a pure culture of the strain was 0.46 mm.; that for strain 205 in set 1 was 1.13 mm. If 89.3% of the nodules in the set with the mixed culture had the former mean length and 10.7% had the latter, the mean length of the nodules in the set should be 0.53. It was actually 0.53 in set 2. A finer check can be obtained from the frequency distribution of nodule lengths in the three sets. Nodules produced by pure cultures of 205 and Coryn have very distinct frequency distributions of length (figure 4). The frequency distribution of nodule lengths to be expected in the mixed culture can be deduced by calculating, for each nodule length, the term $(f_c c + f_a a)/100$, where f_a and f_c are the frequencies of that particular length for strain 205 and Coryn respectively when supplied in pure culture, and

where a and c are the percentages of strains 205 and Coryn estimated from the isolations. The lower diagram in figure 4 shows the expected frequency curve obtained by this calculation together with the distribution actually observed. Both these curves closely resemble that derived from the pure Coryn strain.

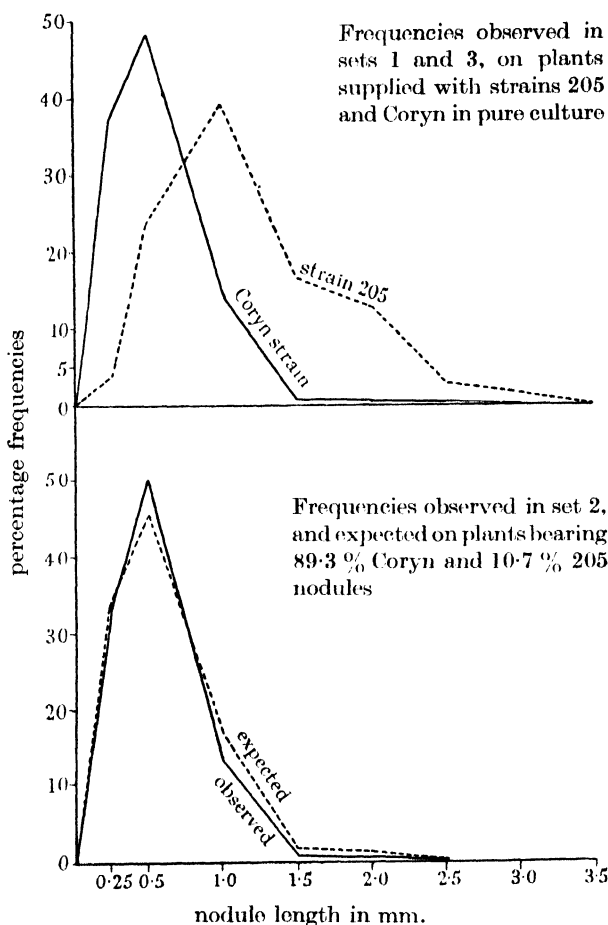


FIGURE 4. Frequency distributions of nodule length in experiment 9, sets 1, 2 and 3.

The large percentage of Coryn nodules in the set supplied with a mixture in about equal numbers of the two strains can largely be accounted for by the very different nodule numbers characteristic of each strain in pure culture, and due to the different degrees of infectivity. Strain 205 by itself produced 16 nodules per plant in set 1 while the Coryn strain pro-

duced 64 in set 3. The figures are in a ratio of 1 : 4. If each strain in the mixture had an equal opportunity to produce its characteristic degree of infection, the numbers of nodules produced by the two strains should be in this proportion, which would give 80 % Coryn nodules in the mixture. The difference between this figure and the percentage 89.3 actually found in set 2 leaves very little to be accounted for by selective competition between the strains either outside or within the plant.

The data obtained with strains A and Coryn are set out in table 7.

TABLE 7. EXPERIMENT 9. CLOVER GROWN IN QUART BOTTLES.
INTERACTION OF STRAINS A AND CORYN

set	no. of plants	strains supplied	mean nodule numbers	standard errors	percentage of Coryn nodules estimated	mean nodule lengths	standard errors	no. of nodules measured
			per plant		from isolations	(mm.)		
4	252	A	11	± 0.6	—	1.09	± 0.01	714
5	87	A + Coryn	17	± 2.0	7.9	1.075	± 0.03	278
3	146	Coryn	64	± 0.8	—	0.46	± 0.02	840

The nodules produced by these two strains are distinguishable by the following characters. (a) Strain A seldom produces more than ten nodules per plant on red clover seedlings grown for 1 month in agar, while Coryn seldom produces less than fifteen. (b) The nodules of strain A more than 1 mm. long are nearly always coloured pink. (c) There is again a marked difference in mean nodule length between the two strains.

Cultures were isolated from 140 nodules taken at random from the set given a mixed inoculum of strains A and Coryn. These cultures were tested on red clover seedlings grown in agar medium. After 1 month's growth the nodules were counted and the isolations were classified according to the number of nodules per plant and the presence or absence of pink pigment. These tests indicated that 92.1 % of the nodules tested contained strain A. This estimate can again be checked by the lengths of nodules in the bottles (see table 7). The mean nodule lengths found in bottles supplied with a pure culture of strains A and Coryn was 1.09 and 0.46 respectively (sets 3 and 4). Hence a mixture of the two strains containing 92.1 % of strain A nodules should have a mean length of 1.043 mm. The figure actually obtained from 278 nodules taken from the bottles in set 5 given the mixed inoculum was 1.075 mm. The frequency distributions of nodule lengths of Coryn and strain A are again characteristic. They are shown in figure 5.

From these can be calculated the distribution of nodule lengths expected in a mixture of 92.1 % A and 7.9 % Coryn nodules. This expected distribution and that actually obtained from the mixed set are compared in the lower diagram in this figure.

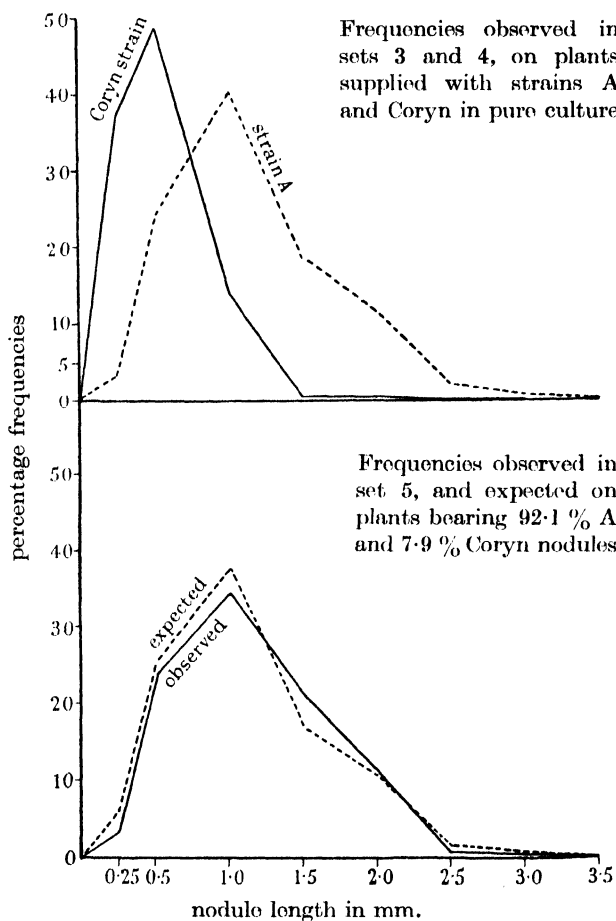


FIGURE 5. Frequency distributions of nodule lengths in experiment 9, sets 3, 4 and 5.

The interaction of strain A with the Coryn strain is in marked contrast with that of strain 205. The mean number of nodules per plant produced by strain A in pure culture was 11 (set 4), while Coryn produced 64 nodules (set 3). These figures are in the ratio of 1 : 5.68, so that if the strains did not interfere with each other but each had an equal chance to produce its characteristic degree of infection, a mixture in equal numbers of the two

strains, as was supplied in set 5, should bear nodules of which 85% should be produced by the Coryn strain. In fact only 7.9% were found to contain Coryn. That this is due to an inhibition of the Coryn strain and not to a stimulation of nodule production by strain A is shown by the small number of nodules per plant (17) produced in the mixed set 5. This is only slightly higher than the number (11) produced by strain A in pure culture.

In experiment 8, plants bearing strain A nodules and transplanted, after washing their roots, into sand containing only Coryn bacteria, subsequently produced as many nodules as did plants that were without nodules at the time of transplanting. Hence the repression of Coryn nodules by strain A in the present experiment cannot be due either to satisfaction of the nodule-bearing capacity of the plant by strain A or to the establishment by it of a specific immunity against infection by the Coryn strain. It can thus be accounted for only through competition between the two strains outside the plant. The different results of the two experiments are associated with the fact that in experiment 8 the two strains were applied in succession whereas in this experiment they were present simultaneously in the sand, initially in equal numbers, so that competition *outside* the plant could take place.

In the case of pea nodule bacteria the dominance in strain 313 in competition with strain 310 was attributed to the more rapid early growth of the former strain. It was therefore to be expected that strain A would also multiply more rapidly than the Coryn strain in the sand surrounding clover roots. The following experiment was made to see whether this was so.

Experiment 10

Pure cultures of strain A and of the Coryn strain were grown in quart milk bottles containing washed sand, using the same technique and the same food solution as in experiment 6. In making the inoculations, a suspension of each strain was counted on a haemocytometer and the two suspensions were standardized so as to contain equal numbers of bacteria. An equal volume of suspension was added to each bottle. Nine replicate bottles were supplied with each strain and all were sown with externally sterilized red clover seed at the rate of about twenty seeds per bottle, on 20 February.

Plate counts on yeast agar were made of the suspensions at the start and from samples of the sand taken from triplicate bottles, after 9, 14 and 18 days. The results are shown in figure 6. By the 9th day the clover seedlings had their first true leaves open and had begun to develop nodules. By this time the numbers of the dominant strain A in the sand were about

double those of the Coryn strain. Thus the strain dominant as regards nodule formation is again, as in pea nodule bacteria, characterized by a more rapid multiplication in the root surroundings than the strain against which it competes with success.

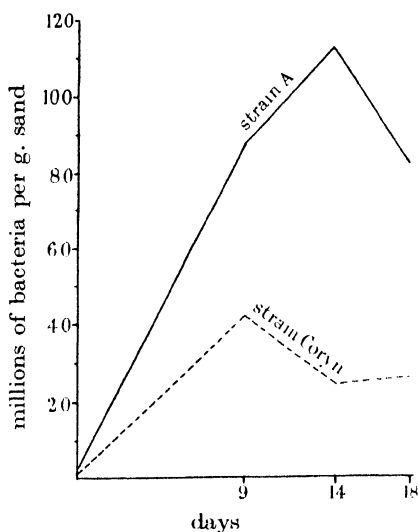


FIGURE 6. Growth of strains A and Coryn in sand surrounding clover roots (experiment 10).

CONCLUSION

The effect of dominance in competition outside the root system would seem to be of paramount importance in determining which of two strains shall contribute most to the production of nodules when both are present in the surroundings of their host root system. This competition, when it occurs, masks the influence of the relative infectivity of the two strains, and shows its effect regardless of whether the first-formed nodules inhibit later ones, since this inhibition is not selective in its action.

There is need for further research on the ecology of nodule bacteria in the surroundings of legume roots. The behaviour of mixed cultures of two or more strains in this environment affords an almost untouched field for work.* In preparing cultures for commercial distribution, it becomes of

* Amongst the points that need investigation is the influence of bacteriophage on strain competition. The cultures used in the present work were tested for bacteriophage with negative results. But the equilibrium between strains growing in mixture might well be disturbed by the presence of a bacteriophage capable of attacking one of them.

practical importance to choose strains that can not only produce nodules beneficial to their host plant, but are also dominant in competition with other strains. The association of this dominance with a high initial growth-rate in sand supplied with an energy source, suggests a convenient laboratory method for assessing the ability of a strain to compete with others.

The intense competition that takes place between closely related strains of bacteria may well have a wider importance, in its application to pathogenic organisms. The work of Greenwood, Bradford Hill, Topley and Wilson (1936) on mouse epidemics has emphasized the distinction between the infectivity and the virulence of pathogenic strains. In *Rhizobium*, Chen and Thornton (1940) have produced evidence that the quantity of nitrogen fixed by a nodule is a function of the growth of the bacteria within it. This is a strain character analogous to virulence in a pathogenic organism. Strains also differ specifically in the number of nodules that they produce, that is, in their infectivity. The present work introduces the further concept of dominance in competition between strains before infection occurs. This competition can be compared with the intra-specific selection that takes place in higher organisms and may well be more acute than competition between more distantly related micro-organisms whose environmental requirements are more dissimilar.

The authors wish to thank Dr H. K. Chen who carried out experiment 8 and who assisted in carrying out experiment 5.

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THE LIMITED NUMBERS OF NODULES PRODUCED ON LEGUMES BY DIFFERENT STRAINS OF *RHIZOBIUM*

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In the field a legume crop usually obtains its nodules from a mixed population of nodule bacteria including a variety of strains doubtless varying in their effectiveness towards the host plant. It is therefore of practical importance to determine what are the factors that determine which strains will infect the plant and in what proportions. Nicol & Thornton (1941) found that an important factor controlling this was the competition that took place between bacteria of different strains *outside* the roots of the host plant. Where one was markedly dominant in competition, this became the determining factor controlling infection. But otherwise the relative infectivity of the strains determined the proportion of the total nodules contributed by each of them.

The numbers of nodules produced on a legume by a given strain of *Rhizobium* will clearly be conditioned by a number of factors, some arising in the root surroundings, others from the general physiology of the host plants, and others more specifically related to the strain of invading bacterium. It is with the latter more specific relationships that the present paper deals. Obvious differences exist between strains of *Rhizobium* not only as regards the mean size but also the mean number of nodules characteristically produced by them under conditions of host-plant cultivation rendered as carefully standardized as possible. These differences might be due to different rates of successful infection,¹ or to the establishment of a limiting equilibrium due to ability of a given strain to produce only a limited number of nodules on a given mass of the root system. If such a limiting equilibrium exists, the number of nodules per gram of root should attain a constant level characteristic of each strain. This number should remain constant on a growing root

¹ A large part of the root hair infections must fail to result in nodules (see McCoy, 1932). The term 'successful infection' is here used to designate infections that result in the formation of nodules.

system though the absolute number of nodules increases with the growth of the roots. On a root system that makes its growth over a short period, the further production of nodules should stop when the roots cease growing or when the specific limiting number of nodules per gram of root has been reached, whichever occurs last.

On this latter type of root system, if the limiting number of nodules per gram of root is quickly reached, the nodules on the young plant will comprise a large fraction of the number finally possible and hence will greatly reduce the formation of further nodules by the same or by a different strain. A number of authors have recorded such an inhibiting effect of early nodules. In particular, Dunham & Baldwin (1931) found that early nodules produced by one strain could entirely inhibit the formation of nodules by a different strain. Nicol & Thornton (1941), however, found that with peas and soy beans the inhibiting effect of the early nodules acted with equal intensity against the same as against a different strain. In this work which was designed to study competition between strains, the second strain was applied to sand already populated with the first, so that the results were complicated by competition between strains outside the plant. To measure the effect of the early nodules upon subsequent infection it is necessary to remove the bacteria, derived from the first inoculum, that remain in the root surroundings, and to transplant the roots into a medium populated only by the second inoculum. Experiments of this kind were made with the object of determining whether the nodule numbers per unit mass of roots attained a limiting equilibrium and how the establishment of this equilibrium affected subsequent infection by the same and by a different strain. These experiments were made with clover, whose root system continues its growth over a long period, and with soy beans, whose roots make most of their growth during early stages of culture.

EXPERIMENTS WITH RED CLOVER, 1939

In this experiment two strains of clover *Rhizobium* were used—the efficient strain 205 obtained from Wisconsin¹ and the inefficient Coryn strain, whose nodules were described by Chen & Thornton (1940). The rates of nodule appearance due to these strains on clover seedlings were found by Nicol & Thornton (1941) to be markedly different, while their

¹ The author's thanks are due to the staff of the Wisconsin Agricultural Experiment Station for supplying cultures of this strain and of the four strains of soy-bean nodule bacteria used in the second experiment, described below.

experiment in which clover was grown in sterilized sand showed that the absolute number of nodules produced in three months' growth by the two strains also differed characteristically.

In the present experiment, Montgomery red clover was sown in wide test-tubes on slopes of agar medium of the following composition:

K_2HPO_4	0.5 g.	NaCl	0.1 g.	$FeCl_3$	0.01 g.
KH_2PO_4	0.5 g.	$Ca_3(PO_4)_2$	2.0 g.	Agar	10 g.
$MgSO_4 \cdot 7H_2O$	0.2 g.	$FePO_4$	0.5 g.	Water	1 l.

The tubes of media were sterilized in the autoclave. Twenty replicates were left sterile, twenty supplied with strain 205 and twenty with the Coryn strain. The bacteria were mixed with the melted agar cooled to $42^\circ C.$ before making the slopes. Two seeds, externally sterilized by immersion for 3 min. in absolute alcohol and for 3 min. in 0.2% $HgCl_2$ and washed with sterile water, were sown at the top of each slope. The seeds were sown on 13 February, and on 27 March the seedlings were removed from the tubes and their nodules counted. They were then replanted in small pots each containing 3 kg. of nitrogen-deficient sand, sterilized by blowing superheated steam through each pot for half an hour. 175 ml. of the following sterilized food solution was added to each pot:

K_2SO_4	0.9 g.	$FeCl_3$	0.02 g.
K_2HPO_4	0.5 g.	Boric acid	0.02 g.
$CaH_2(PO_4)_2 \cdot 4H_2O$	0.5 g.	$MnSO_4$	0.02 g.
$MgSO_4 \cdot 7H_2O$	0.5 g.	Tap water	990 ml.
NaCl	0.5 g.	Lucerne root extract	10 ml.

Twenty replicate pots were supplied with a heavy inoculum of strain 205 and twenty with one of the Coryn strain, the bacteria being mixed with the food solution before addition. One seedling bearing strain 205 nodules, one bearing Coryn nodules and two plants without nodules were planted in each pot, each plant being separately labelled. On 10 July the roots were washed, the nodules on each plant were counted and the dry weights of individual root taken. The results are shown in Table 1.¹ The experiment was so designed as to test whether any of the following factors had any effect upon the final nodule numbers per gram of root:

¹ The nodules per gram of root were separately calculated for each plant and the means of the figures so obtained are those shown in the table. They differ from those derivable from the mean nodule numbers (column 5) and the mean root weights (column 6). The same process was followed for the corresponding figures in Table 3.

(1) time at which the bacteria were first applied; (2) size of the root system as modified by the efficient strain applied at seeding time; (3) a possible inhibiting action of the early nodules against the same or a different strain.

Table 1. *Effect of strain of Rhizobium on nodule numbers in red clover*

Set	Strain applied		Mean nodule numbers per plant		Final root dry wt. mg.	Final nodules per g. root	n
	At sowing time	After trans-planting	When transplanted	At end	Means per plant		
1	—	Coryn	—	357.2 ± 42.8	118	3402.4 ± 475.8	17
2	Coryn	Coryn	38.7 ± 4.4	385.5 ± 74.0	163	2770.9 ± 558.3	10
3	205	Coryn	7.3 ± 0.8	904.1 ± 181.4	381	2273.7 ± 360.9	14
4	—	205	—	182.1 ± 30.8	355	544.8 ± 56.8	15
5	Coryn	205	50.0 ± 1.4	169.3 ± 38.4	239	694.5 ± 101.3	10
6	205	205	9.1 ± 1.5	331.1 ± 50.7	573	652.6 ± 121.3	14

At the time of planting out, seedlings that had grown for 6 weeks on agar already showed differences in nodule numbers characteristic of the strain supplied at seeding time (column 4). When removed from the agar the seedling root system showed no differences in size according to the culture supplied—the efficient nodules not having had time to produce increased growth.

After transplanting into the pots some plants died. The numbers surviving can be deduced from the degrees of freedom, *n*, shown in the last column. These made considerable growth before harvest with a large increase in nodule numbers. The final root weights shown in column 6 were much increased where 205 nodules, effective in nitrogen fixation, had developed on the seedling while growing in agar. This appears in comparing set 1 with 3 and set 4 with 6.

The absolute number of nodules at the end of the experiment (column 5) were not significantly affected by the presence of Coryn nodules on the seedlings but the presence of 205 nodules at the time of transplanting greatly increased subsequent nodule formation by either strain. This effect was in fact due to the enlargement of the root system resulting from nitrogen fixation by the early-formed efficient strain 205 nodules.

The nodules per gram of root (column 7) show no significant differences between sets receiving different treatments in their early growth but later grown in pots supplied with the same strain. Thus the time at which the bacteria were first supplied to the roots was without final effect on the nodules per gram of root. Nor were there very large

differences in size of the root system produced by the early formed 205 nodules. There were, on the other hand, large differences in the mean number nodules per gram of root according to the strain in the sand which was in contact with the root system during the period when it made most of its growth. This mean number reached a definite limit characteristic of the strain present in the sand. This limit was apparently attained quite early in the plant's growth. The mean figure for plants grown in pots containing Coryn bacteria was 2816, and that for plants in pots containing strain 205, only 631 nodules per gram of root. These figures are in the ratio of 4.6 : 1. This ratio can be compared with that between the actual nodule numbers developed by the two strains in agar (column 4), because during this early period the size of the root systems was similar in all sets. The Coryn strain developed a mean of 44.4 nodules per seedling, and strain 205 a mean of 8.2, at the time of transplanting. These figures are in the ratio of 5.4 : 1. So that the two strains produced nodules whose numbers per unit of the root system were in approximately the same ratio both on seedling roots grown in agar and subsequently on plants grown in pots of sand. Thus the limit of infection for a root system of given size is characteristic of each strain and is quickly reached. But the absolute nodule numbers of nodules increased *pari passu* within the growth of the root system, which in clover continues over a long period. This explains why the presence of nodules on the seedling did not stop further nodule formation, which took place on a growing root system, and why the final number of nodules per gram of root was that characteristic of the second applied strain, which was in contact with the root system while this was making most of its growth.

The following experiment, similar in general design to the first, was made with soy beans, whose root system makes most of its growth when the plant is quite young. It was designed to determine what specific limits of nodule numbers per gram of root were possessed by four strains of soy bean *Rhizobium* and to test the influence of the early nodules upon later infection by the same and by different strains.

EXPERIMENT WITH SOY BEANS, 1939

Soy beans were grown in glazed earthenware pots each containing 12 kg. of sand and 1 l. of food solution similar in composition to that used in the first experiment. Five seeds externally sterilized were sown in

each pot on 21 June. Eight replicate pots were left uninoculated and eight each were supplied with each of the following strains of *Rhizobium*:

Wisconsin 501	} Effective
„ 505	
„ 502	} Ineffective
„ 507	

The plants were grown for 9 weeks and their roots were thoroughly washed and the nodules counted. They were then replanted in the pots in such a way that each pot whose sand contained an effective strain (501 or 505) received one plant bearing nodules produced by the same strain, one plant bearing nodules produced by each of the ineffective strains and one uninoculated plant. Similarly each pot whose sand contained an ineffective strain (502 or 507) received one plant bearing nodules produced by the same strain, one plant bearing nodules produced by each of the effective strains and one plant without nodules. Each plant was separately labelled. The scheme of transplanting is shown in Table 2. After a further 14 weeks' growth the nodules were recounted

Table 2. *Soy-bean experiment, scheme of transplanting*

Plants with nodules, when transplanted, of strain	Transplanted into pots whose sand contained strains			
	501 Set	502 Set	505 Set	507 Set
501	1	2	—	3
502	4	5	6	—
505	—	7	8	9
507	10	—	11	12
No nodules	13	14	15	16

and dry weights of the roots were taken. The results are shown in Table 3. The plants which bore nodules produced by strains 501, 502 or 505 before transplanting did not show any significant increase in nodule numbers after transplanting (sets 1-9, columns 4 and 5). Thus the limit of nodule numbers attainable on the root systems in these sets had been reached within the first 9 weeks' growth. The number of nodules per gram of root was specific to the strain of *Rhizobia* (column 7). The mean number of nodules per gram of root in sets 4, 5 and 6 which bore nodules produced by strain 502, was 284.7, a figure significantly higher than the mean numbers, 191.7 and 163.4 of the sets whose nodules were produced by strains 501 and 505 respectively (sets 1-3 and 7-9).

The plants without nodules at the time of transplanting made considerably greater root growth during the second growth period, probably

Table 3. *Effect of strain of Rhizobium on nodule numbers in soy beans*

Set	Strain applied		Mean nodule numbers per plant		Final root dry wt. mg. Mean per plant	Final nodules per g. root	n
	At sowing time	After transplanting	When trans-planted	At end			
1	501	501	17.7	16.1 \pm 2.7	94	206.3 \pm 30.2	9
2	501	502	17.2	18.5 \pm 1.5	162	149.8 \pm 38.6	4
3	501	507	22.4	22.6 \pm 3.4	144	219.1 \pm 68.4	6
	501	Mean				191.7 \pm 25.9	21
4	502	501	29.1	29.4 \pm 4.8	123	269.1 \pm 44.7	8
5	502	502	35.9	37.8 \pm 4.4	158	284.3 \pm 38.1	7
6	502	505	36.3	36.5 \pm 6.1	131	300.8 \pm 46.9	9
	502	Mean				284.7 \pm 24.6	26
7	505	502	18.9	21.0 \pm 1.7	170	134.4 \pm 20.9	6
8	505	505	22.5	19.6 \pm 2.0	114	184.1 \pm 29.9	7
9	505	507	18.4	19.3 \pm 2.0	185	171.7 \pm 50.6	6
	505	Mean				163.4 \pm 19.6	21
10	507	501	28.0	34.6 \pm 4.7	77	451.0 \pm 56.5	6
11	507	505	29.6	44.6 \pm 8.9	101	446.0 \pm 49.4	7
12	507	507	32.1	56.6 \pm 10.1	141	436.2 \pm 53.5	8
	507	Mean				444.4 \pm 29.4	23
13	—	501	—	30.9 \pm 7.4	179	211.4 \pm 26.0	7
14	—	502	—	70.3 \pm 13.4	303	233.0 \pm 39.7	8
15	—	505	—	43.0 \pm 8.8	244	186.4 \pm 27.4	7
16	—	507	—	57.0 \pm 13.4	253	225.3 \pm 43.6	7

because they were smaller at the time of transplanting and suffered less check. These plants in sets 13, 14 and 15, planted in sand containing bacteria of strains 501, 502 and 505 respectively, developed nodules whose numbers per gram of root did not differ significantly from those on plants that had received the corresponding strain at the time of sowing (compare sets 1 and 13, 5 and 14, 8 and 15). Thus the specific limit of nodules per unit mass of root system was attained regardless of the total mass of the root system, which varied widely, or the time at which the infection took place. This latter point shows that the number of nodules is determined by the size of the root system and not vice versa, since most of the root growth in sets 13, 14 and 15 took place before the plants had developed any nodules. Strain 507 has a much higher level of nodule numbers than the other three strains. The mean final nodule numbers per gram of root for sets 10, 11 and 12 was 444.4, a figure significantly higher than that for any other set or group of sets. This high figure was not reached during the period of 14 weeks' growth in set 16 which first received the bacteria at the time of transplanting. Nor was the full number of nodules reached during the first 9 weeks of seedling growth in sets 10, 11 and 12, which developed more nodules after transplanting. In sets 10 and 11 these additional nodules were in fact

produced by the strains 501 and 505 respectively as was shown by examining the nodules.¹ These later-formed nodules were comparatively few and the number of nodules per gram of root finally reached was that characteristic of strain 507. The figures for sets 10 and 11, 451 and 446, do not differ significantly from that of 436.2 for set 12 which received strain 507 both at sowing time and after transplanting.

DISCUSSION

In the experiments described above the number of nodules n divided by the dry weight of the roots m was found to reach a limiting figure that was constant and specific for each strain of *Rhizobium*,

$$n = mk.$$

If a plant's roots are exposed in succession to pure cultures of two strains of *Rhizobium* having the limiting constants k_1 and k_2 and if each strain is allowed time to reach its limit, the number of nodules n_1 produced by the first strain will be $m_1 k_1$ where m_1 is the mass of the roots developed while in contact with this strain. On the simplest supposition, the number n_2 produced by the second strain will be $m_2 k_2$ where m_2 is the additional mass of roots developed in contact with it. The total nodules developed by the two strains will therefore be

$$n_1 + n_2 = m_1 k_1 + m_2 k_2.$$

In the experiment with clover, nearly all the root growth took place after transplanting so that m_2 was very large relatively to m_1 . Consequently the number of nodules was determined by k_2 and was that characteristic of the second-applied strain. In the soy bean experiment, all or nearly all the root growth took place in the presence of the first-applied strain, whose specific constant, k_1 , determined the limit of nodule numbers reached.

It would be interesting to investigate the condition where the host is removed into the presence of the second strain before the first strain had reached its limit of infection for a given mass of roots, and, to discover to what extent the first strain can then impose its specific limit on further nodule formation in these same roots by the second strain. Thus if the number of nodules produced by the first strain, $n_1 = m_1 k_1 - x$, would the number x , produced by the second strain, be determined by

¹ Nodules produced by the effective strains 501 and 505 have soft reddish centres easily distinguishable in hand sections from the hard whitish centres of the ineffective nodules produced by strain 507 (see Nicol & Thornton, 1941).

the constant k_1 or by a constant k_2 specific to the second strain? The answer to this question might throw light on the mechanism of nodule limitation. The evidence from sets 10 and 11 in the soy bean experiment suggests the continued operation of the constant k_1 specific to the first strain, but this evidence is insufficient to form any basis for discussion.

SUMMARY AND ABSTRACT

Pot experiments were made with red clover and with soy beans to determine how far the number of nodules developed was a specific character of the strain of *Rhizobium* supplied.

The number of nodules per gram of root was found to reach a limit specific to each strain. This limiting equilibrium was attained regardless of the size of the root system or the age of plant at which the culture was first supplied, provided enough time were allowed for the limit to be reached.

When two different strains were applied to the root surroundings in succession, the final number of nodules was determined by the limit specific to the strain in contact with the roots while these were making most of their growth. In clover this was the second and in soy beans the first applied strain.

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NOTE CONCERNING AUTHORSHIP

The work described in this paper was carried out by Dr Chen shortly before his departure for Central China. Some difficulty in communication due to wars has made it necessary for the undernamed to write the paper from Dr Chen's notes and data without his having the opportunity to see it before publication. The writer thinks he has drawn conclusions from the data in agreement with Dr Chen's opinions, but he accepts full responsibility for these conclusions and for the actual writing, although credit for the work is due solely to Dr Chen.

H. G. THORNTON.

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SELECTIVITY IN BACTERIAL FOOD BY SOIL AMOEBAE IN PURE MIXED CULTURE AND IN STERILIZED SOIL

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(With Plate 4 and 3 Text-figures)

EARLIER workers succeeded in getting "pure mixed cultures" of amoebae by feeding them on dead or living bacteria or yeasts. Oehler (1916, 1924*a, b*) found that amoebae eat more readily the Gram-negative species of bacteria than the Gram-positive species, and that of five species of amoebae some could grow on dead bacteria and yeasts, killed by heating, while others could grow only on living bacteria and yeasts. Severtzova (1928) studied the food relationships of soil amoebae with twenty-six species of soil bacteria, twelve moulds, four yeasts and two actinomycetes. She concluded: "If we examine the list of bacteria we find that neither the presence of proteolytic ferment, nor the ability of denitrification, of nitrogen fixation and of ammonification, nor the capacity of motion, nor the presence of pigmentation and, certainly, not the relation to Gram staining can account for the selective action by the amoebae. The small, motile, non-spore-bearing bacteria as well as the small immotile cocci seem to represent the most suitable food the amoebae can find in the soil." Among the edible species of bacteria some were more readily accepted by amoebae than others. Among the spore-forming bacilli the vegetative forms were preferred to the spores.

Cutler & Crump (1927, 1935), using *Hartmanella hyalina*, found that different species of bacteria appear to have different nutritive values. The bacterial species "YB" and "SE" are of the same size and shape, yet their nutritive value, judging by the rate of reproduction of the amoebae feeding on them, is quite different. They concluded that this is a true feeding effect because they did not observe that the waste products of the bacteria had a bad effect on the amoebic growth. Rice (1935, 1938), using *Flabellula mira* and three other species of marine amoebae, observed that whether the bacteria were Gram-positive or Gram-negative made little difference to their utilization as food by the amoebae. Of ten species of bacteria, *Serratia ruber* was untouched by the amoebae; some of the species were eaten by all the amoebae, while others were eaten by some species of amoebae and were untouched by others.

A. SELECTIVITY IN BACTERIAL FOOD BY SOIL AMOEBAE IN "PURE MIXED" CULTURE

Material and methods

A small and a larger species of soil amoebae belonging to the *Limax* group were obtained from Barnfield farmyard manured soil (Rothamsted Experimental Station, Harpenden). A fragment of soil was placed in the centre of a bacterial circle on an agar plate and incubated at 20° C. When, within a few days, the amoebae had moved outside the bacterial circle, some of them were put again into the centre of a freshly prepared bacterial circle. This process was repeated till a pure amoebic culture was obtained. To get a pure line culture from a single amoeba, it was washed several times in

sterilized saline and then placed in the centre of a small bacterial circle of the given species as food. Micropipettes were used for isolating the amoebae and transferring them from one washing solution to the next.

A method for studying food selection by amoebae

Amoebae migrate in all directions on the plate when they are placed either in the centre of bacterial circles or stars. This difficulty was overcome as follows: pieces of hard glass tube are pulled into thin tubes of uniform size, broken into small pieces, and arranged inside Petri dishes in as many radii as desired (Text-fig. 1 and Pl. 4). The Petri dishes with the tubes are sterilized and agar is poured between the tubes with a fine pipette. The tubes are arranged with small sterile forceps, and stick to their positions when the agar has solidified. Bacterial streaks are made on the agar and amoebae are placed in the centre of them.

Throughout the experiments 2% agar containing 5 g./l. NaCl was used, this giving better results than nutrient agar because the bacteria are not able to multiply as quickly. The species of bacteria used and the sources from which they were obtained are given in Table 1. In all the feeding experiments bacterial cultures of 3–10 days were used. The temperature of incubation was 20°C. For a comparative study of the preference in food, bacterial cultures of the same age were always used.

Observations

Species of Aerobacter preferred by amoebae.

Five strains of *Aerobacter* were used (1912, 08, 07, 1734 and 2006). They show identical morphology, and more or less similar physiological reactions (Table 1). A series of plates was made by the method described and amoebae were inoculated in the centre of the bacterial stars. Amoebae do not show extreme difference in their preference towards the *Aerobacters* but they destroy some strains in larger numbers than others, and move along the bacterial radii in those cases much more quickly. Species 1912 is the food preferred most by the amoebae, and the others are accepted in the following order 08, 07, 1734 and 2006 (Pl. 4, fig. 1).

Various degrees of selectivity in bacterial food as exhibited by the soil amoebae.

The seventeen kinds of bacteria used (Table 1) may be grouped according to their suitability as food for the amoebae: λ T 20, 1912, 08, 07, 1734, 2006 and S 21 are eaten readily by the amoebae, N 16(i) and 4045 are eaten slowly, and 0312, 0746, 2881, 5654, 5431, 4022, 4031 and R are either untouched, or are eaten very slightly if they are the only food available.

In selectivity experiments bacteria were inoculated along the radii, each radius consisted of a different kind of bacterium, and the amoebae were inoculated in the centre. The preference for one type of food as opposed to another was judged by the amount of bacteria destroyed along each radius in a given time. All the experiments were repeated several times before final conclusions were arrived at. In each experiment both edible and non-edible bacteria were used. Among the readily accepted food types the amoebae eat most readily species λ T 20 and move along this bacterial radius much more quickly than along the others in a given time. The other types of bacteria are preferred by the amoebae in the following order, 1912, S 21, 08, 07, 1734 and 2006. The choice made by the amoebae among the bacteria 4045, N 16(i), R, 4022, 4031, 5431, 5654, 0312, 0746 and 2881 is as follows: 2881, 0312, 0746, 5654, 5431 are never touched even when they are the only available food; R, 4022 and 4031 are on rare occasions very slightly eaten by the small amoeba used in these experiments but not by the larger type; 4045 and N 16(i) are slowly

TABLE 1. *Sources and characters of bacteria used in experiments*

Strain	Motility	Morphology	Liquefaction	Gelatine	Milk	Dextrose	Laevulose	Sucrose	Lactose	Dulcitol	Nitrate reduction	Indol	Locality	Colour	Gram staining
07	o	Short rod	o	Thread	Acid curd R.L.	G 4.0	G 4.6	G <4.0	G <4.0	6.9	+++	++	Milk waste	Milky white	-
08	+	Short rod	o	Thread	Acid slow curd R.L.	G 5.8	G 5.5	G 5.5	G 5.3	6.9	+++	o	Milk waste	Milky white	-
1734	o	Short rod	o	Thread	Acid curd R.L.	G 4.7	G 4.3	G 4.2	G 4.4	6.9	+++	Trace	Milk waste	Milky white	-
1912	o	Short rod	o	Thread	Acid curd R.L.	G 4.7	G 4.7	G 4.6	G 4.1	6.9	+++	o	Milk waste	Milky white	-
2006	+	Short rod	o	Thread	Acid curd R.L.	G 5.6	G 5.9	G 4.3	G <4.0	4.4	+++	o	Milk waste	Milky white	-
S 21	o	Very small rod	+	Cup	R.L.	6.5	6.3	7.1	6.9	7.0	+++	Trace	Barnfield F.Y.M.	Milky white	-
N 16 (i)	o	Sarcina	o	Thread	o	6.9	6.9	6.9	6.9	7.0	o	o	Sugar effluent	Citron yellow	+
λT 20	+	Cocci	+	Saucer	o	6.9	6.9	6.9	6.9	7.0	o	o	Sugar effluent	Yellow ochre	+
2881	+	Very short rod	+	Infundibular	Acid curd	<4.0	<4.0	<4.0	6.6	6.7	+++	+	Milk waste	Eugenia red	-
0312	+	Short to medium rod, pairs: short chains	o	Thread	(Alk.)	6.6	.	.	6.6	7.0	o	o	Milk waste	Tawny	-
0746	o	Thin rod	o	Thread	Alk.	6.7	7.1	6.9	6.9	7.0	+++	o	Milk waste	Flesh ochre	-
4022	o	Very small rod	o	Thread	R.L.	5.2	5.5	5.4	6.6	7.0	+	o	Barnfield F.Y.M.	Citron yellow	-
4031	o	Medium rod: single and pairs	o	Thread	o	6.7	6.4	6.9	6.7	7.0	o	o	Barnfield F.Y.M.	Antimony yellow	+
4045	o	Sarcina	+	Saccate	o	6.8	6.9	6.9	6.9	7.0	o	o	Broadbalk Plot 3	Citron yellow	+
5431	+	Medium rod	o	Thread	Pept.	6.3	5.5	5.7	6.6	7.0	+++	o	Barnfield F.Y.M.	Blackish violet	-
5654	o	Small oval rod	+	Infundibular	Curd turns pink	G 4.2	G 4.5	<4.0	6.0	7.0	+++	o	Park Grass Plot 2	Spinel red	-
R (Radiobacter) (N.C.T.C. 1376)	+	Small rod	o	Thread	(Alk.)	6.5	6.3	6.3	6.7	6.8	+++	o	Soil	Milky white	-

eaten, but the amoebae prefer 4045 to N 16(i). It has been found that both species of amoebae prefer the same kind of food, to the same degree, except in the case of bacteria R, 4022 and 4031.

When the amoebae are inoculated in the middle of bacterial stars consisting of both edible and non-edible bacteria, they move in large numbers in all directions in search of food. After reaching the suitable food supply they eat, multiply and move along the bacterial stars. When they reach the non-edible food supply, they either encyst within a short time or move along these radii to some distance without destroying the bacteria, and finally encyst. It is possible also that some of them die.

Selectivity of food by soil amoebae when two kinds of bacteria are arranged side by side.

Two streaks, one of edible and the other of non-edible bacteria, were made touching each other (Pl. 4, figs. 3, 4): the amoebae were inoculated in the centre of the bacterial streaks. The amoebae eat the edible type of bacteria and leave the non-edible ones. Pl. 4, fig. 4 shows that bacteria 4031 is also slightly eaten by the amoebae. In the presence of species 2881 (*B. prodigiosus*), however, the amoebae are able to eat the readily edible species S 21 only slightly (Pl. 4, fig. 3). In some cases it happens that, in the presence of *B. prodigiosus*, amoebae eat a little edible food for a day or two and then encyst. Pl. 4, figs. 3, 4 show clearly that the amoebae do not destroy the bacterial species 4022, 0312, 5654, 2881, R, 5431 and 0746 even when they are present side by side with edible types and touching each other. The cause of the difference between 2881 and the other non-edible species will be discussed later.

Selectivity of food by amoebae among different kinds of edible and non-edible types of food supply.

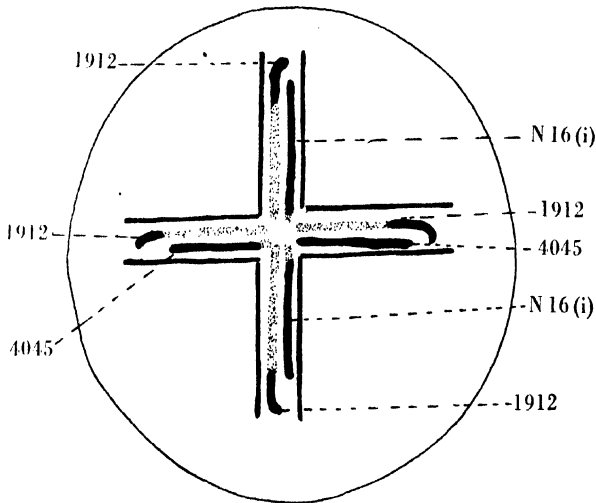
Bacteria were arranged as shown in Pl. 4, figs. 2, 5 and 6, and amoebae were inoculated in the centre and allowed to move in all directions: the photographs show that the amoebae although moving in masses in between the non-edible food supply, ate only the edible food and left the bacterial species 5431, 4022, 0746, R, 2881 and 5654.

Amoebae either encyst when they reach unfavourable food, or they move on in search of a favourable food supply when they feed and multiply. Pl. 4, fig. 5 was taken 5 days after inoculating with amoebae: some of the edible bacteria are either untouched by the amoebae or eaten only very slightly. Pl. 4, fig. 6 was taken 8 days after the inoculation of the amoebae: in this the amoebae have eaten practically all the edible kinds of bacteria, and have left the non-edible kinds.

Preference in food by amoebae among readily accepted and non-readily accepted food supply.

Readily accepted and less readily accepted bacteria were arranged as shown in Text-fig. 1 and amoebae were placed in the centre touching the bacteria 1912. In most cases the amoebae ate first the readily accepted food 1912, and when this food was finished the amoebae began to eat the less readily accepted food, 4045 and N 16(i). Sometimes the amoebae ate very slightly bacteria N 16(i) and 4045 even when there was plenty of readily accepted food supply. If amoebae are cultured on bacteria 4045 or on N 16(i) for some time and then inoculated in the centre of the bacterial streaks as shown in Text-fig. 1, they

start eating both the bacteria (4045 and 1912) at the same time. That is, if amoebae are accustomed to eat less readily accepted bacteria they do not show the preference for eating the most readily accepted food first and later the less readily accepted ones as described above.



Text-fig. 1.

Why are some bacteria readily accepted, other less readily, and the rest untouched by the amoebae?

To test the presence of exo-toxins among the non-edible bacteria, they were plated on agar slopes and allowed to grow thickly for 10–15 days. The bacteria were then scraped off and the agar was melted and poured into sterile Petri dishes. In each Petri dish containing the exo-toxin of one kind of bacteria several bacterial circles of edible bacteria were made, and amoebae were inoculated in the centre. After a few days the amoebae ate the food supplied to them, and destroyed the edible kinds of bacteria in large numbers. The same result was obtained, when the two types of bacteria were put together touching each other. These two experiments clearly show that the non-edible types of bacteria used (except 2881) do not produce exo-toxin in sufficient quantity to prevent the amoebae from eating the edible food supply. In the presence of the exo-toxin of bacteria 2881 the amoebae are either unable to eat the edible food supply or they eat it very slightly and finally encyst or die. This is the case whether the agar containing the exo-toxin is used or the bacteria 2881 is put side by side with an edible type of bacteria, and touching it.

To test the presence of endo-toxins in non-edible bacteria, they were crushed in a glass bacterial mill with normal salt solution, filtered through an L₃ candle, and the filtrates were collected. A series of bacterial circles consisting of edible kinds of bacteria was made on agar plates, and one or two drops of liquid containing the products of the crushed bacteria were added to each of the bacterial circles: the amoebae were inoculated in the centre. After 2–3 days the amoebae had destroyed the edible bacteria in very large numbers, showing that the non-edible bacteria did not contain endo-toxin. Sometimes it is difficult to filter proteins through bacterial candles, and it may be possible that endo-toxin of the

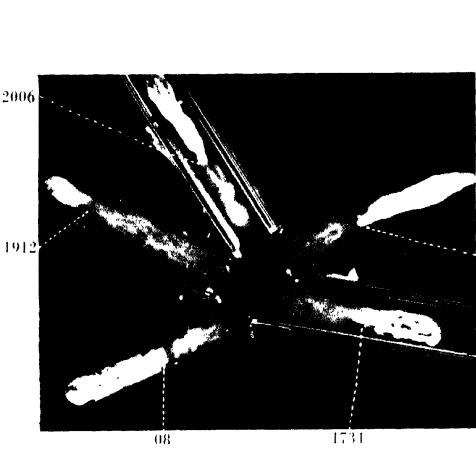


Fig. 1.

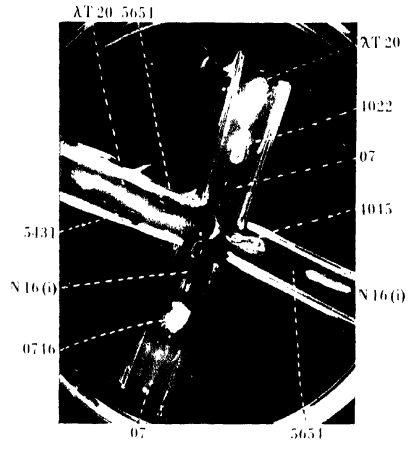


Fig. 2.

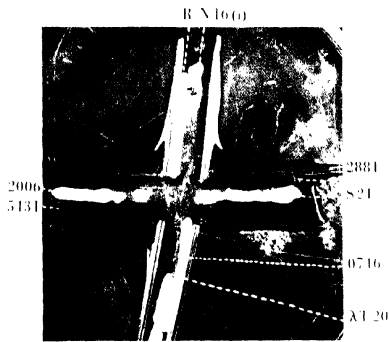


Fig. 3.

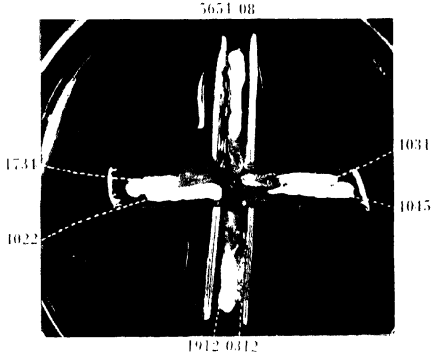


Fig. 4.

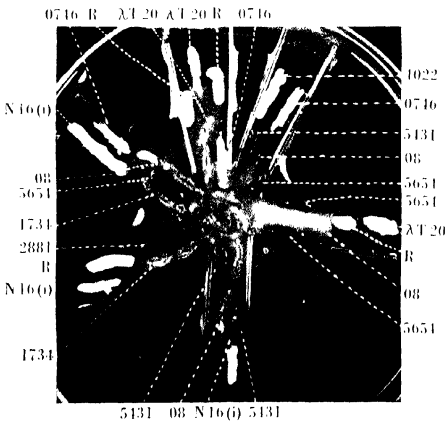


Fig. 5.

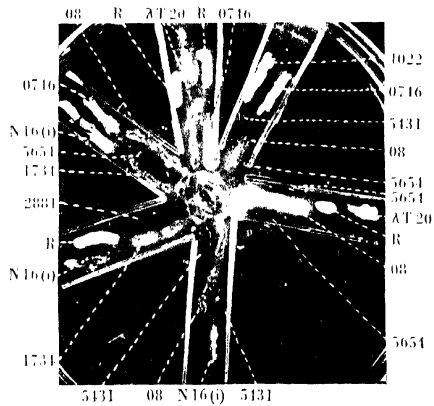


Fig. 6.

bacteria does not reach the filtrate. To test this point the non-filtered crushed bacteria were used in the above experiments: the result was the same. It may be possible that the quantity of endo-toxin is so little as to have no effect on the amoebae.

It is interesting to note that the crushed product or the crushed and filtered product of bacteria 2881 (*B. prodigiosus*) has no effect in preventing amoebae from eating edible food.

It is clear from Table 1 that these amoebae have no special preference for the Gram-negative or the Gram-positive bacteria. The bacteria 07, 08, 1734, 1912, 2006 and S21 are Gram-negative and are readily eaten by amoebae while 2881, 0312, 0746, 4022, 5431, 5654 and R which also are Gram-negative are not touched by the amoebae. The same is true for Gram-positive bacteria, i.e. some of them are eaten by the amoebae while others are not. A large number of Gram-positive bacteria were not used, but it seems certain that, contrary to the claims of Oehler (1916, 1924 *a, b*), the amoebae have no special preference for Gram-negative bacteria.

The majority of the bacteria used were either short, very small, or medium-sized rods (Table 1). Amoebae eat some of these bacteria and leave the others, showing that the size of the bacteria has no evident relationship with edibility. The same is true in the case of motile and non-motile bacteria. Pigmentation in bacteria does not seem to be related to their edibility; of the pigmented bacteria, shown in Table 1, λT 20 is eaten readily by the amoebae, N 16(i) and 4045 are eaten slowly, and the rest are either very slightly eaten sometimes (4022, 4031) or completely untouched (5654, 5431, 0746, 0312, 2881).

B. SELECTIVITY IN BACTERIAL FOOD BY SOIL AMOEBAE IN STERILIZED SOIL

Methods

Soil from the plot in Barnfield, annually manured with 14 tons of farmyard manure per acre, was selected as experimental material. It is a heavy clay soil with a pH from 7.1 to 7.3. The soil was air-dried, powdered and sieved. Tubes containing 20 g. of soil were autoclaved at 15 lb. pressure for 1 hr. on three successive days. Tests showed that this method sterilized the soil with very little change in the pH value. 300 g. portions of soil were placed in large sterile Petri dishes. The inocula used were:

- (1) Bacterial species 5654 alone.
- (2) Bacterial species 5654 + bacterial species 4045.
- (3) Bacterial species 4045 alone.
- (4) Bacterial species 4045 + bacterial species 5654 + one species of soil amoeba.

The amoebae were cultured on species 4045 for 4-6 months, and tests showed that in the culture of amoebae only that species was present. In a throat spray fitted with the finest nozzle obtainable the suspensions of bacteria and amoebae were made in saline. In one spray there was the suspension of species 4045; in a second, of species 5654; and in a third, of amoebae grown on species 4045. The amoebae were mostly in the cystic condition, and there were very few bacteria (4045) in the suspension containing the cysts.

Sterile tap water was sprayed on the soil contained in the large Petri dishes, followed by the sprays of bacteria and amoebae. By this method it is not possible to inoculate the bacteria into the soils in equal numbers, but care was taken to ensure that approximately the same numbers of bacteria were inoculated in the different Petri dishes containing the soils. The water content of the soil was 25 %. Every 3-4 days sterile tap water was sprayed on to the soils to bring their moisture content to 25 %. The Petri dishes were kept in an incubator at room temperature. The numbers of bacteria were counted on the next day after inoculating them in the soil and further counts were taken as shown in Table 2. In the beginning of the experiment it was not possible to count the numbers of bacteria every day owing to the disturbance created by the outbreak of the war.

To count the numbers of bacteria 10 g. of soil was taken from each dish, diluted to 1/500,000 in sterile saline and plated out: from each of the final dilutions five plates were poured, and the number

TABLE 2. *The numbers of bacteria are given in millions/g. of soil*

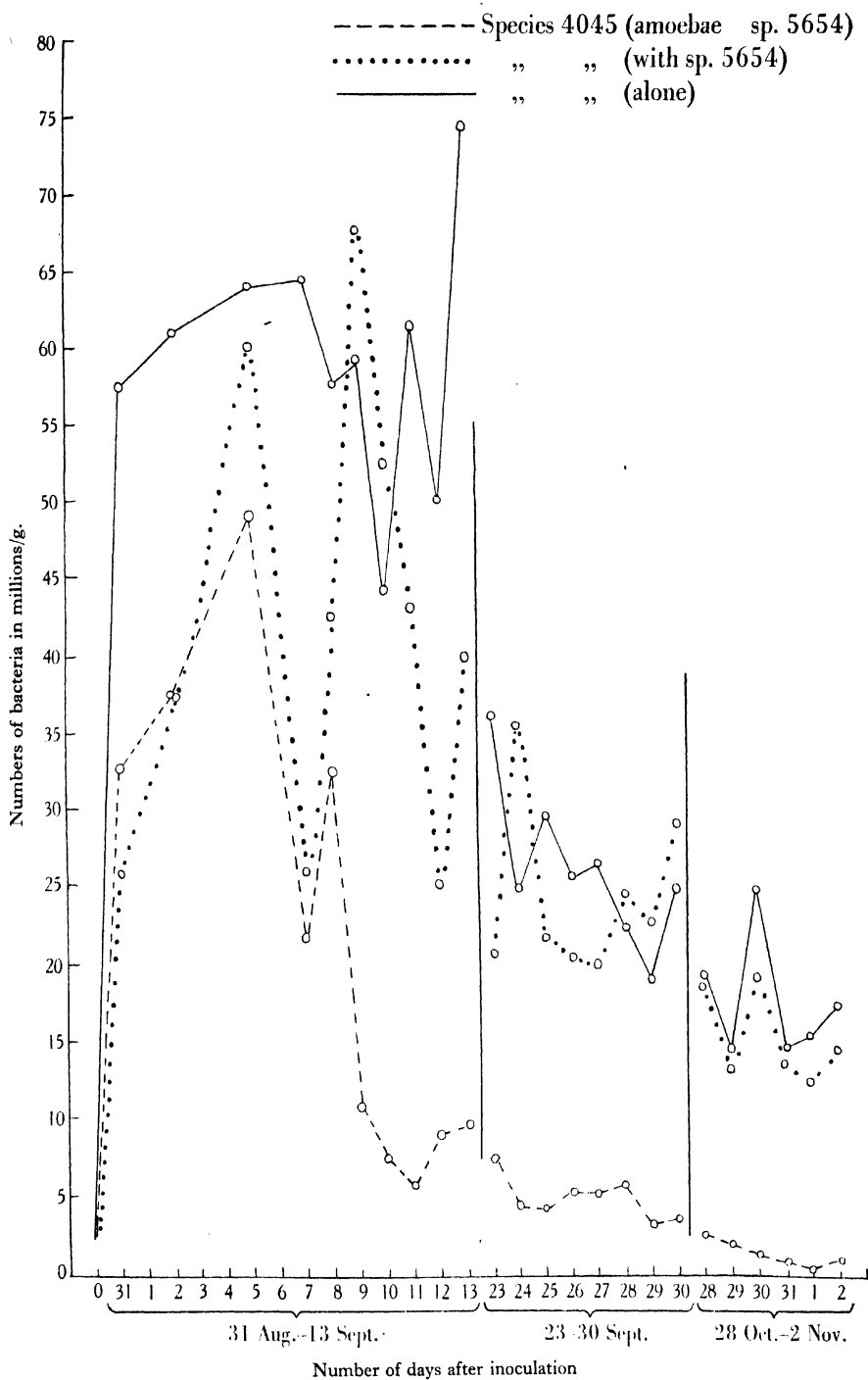
Date	A bacteria + amoebae		B control bacteria only		C control bacteria only	D control bacteria only
	5654	4045	5654	4045	5654	4045
1939						
31 Aug.	231.5	32.5	204.5	26.0	239.0	57.5
2 Sept.	170.0	37.5	187.5	37.5	253.7	61.2
5 "	186.2	48.7	275.0	60.0	256.2	63.7
7 "	97.2	22.0	172.5	26.2	240.0	64.0
8 "	70.7	32.5	121.7	42.5	122.2	57.5
9 "	52.2	10.5	107.5	67.5	176.2	58.7
10 "	28.0	7.5	58.2	52.5	87.5	43.7
11 "	75.0	6.2	119.7	43.2	227.0	61.5
12 "	54.7	9.0	92.2	25.2	139.0	50.2
13 "	118.2	9.5	176.7	40.0	162.5	74.0
23 "	95.5	7.5	112.0	21.5	109.0	36.5
24 "	86.5	4.5	118.5	36.0	105.5	25.0
25 "	91.2	4.2	115.0	22.0	142.0	29.5
26 "	67.5	5.5	107.5	21.0	152.5	26.0
27 "	57.2	5.5	116.0	20.0	97.0	27.0
28 "	43.0	6.5	81.5	24.5	100.0	22.5
29 "	43.2	3.2	86.0	23.0	80.0	19.0
30 "	95.0	3.7	99.0	29.0	72.5	25.0
28 Oct.	98.0	2.5	92.5	18.5	114.5	19.0
29 "	90.5	2.0	86.5	13.5	94.0	14.0
30 "	99.5	1.6	116.0	19.5	145.0	25.0
31 "	93.5	1.0	62.0	13.5	76.0	14.5
1 Nov.	62.0	0.8	56.0	12.5	76.5	15.5
2 "	78.0	1.1	75.5	14.5	73.0	17.5

of bacteria in 1 g. of the soil was calculated. The Petri dishes were incubated for 2-3 weeks at room temperature. When the number of bacteria 4045 became low in the soil, five extra plates were poured from the dilution of 1/50,000 to count the number of that bacteria. The characteristics of the two types of bacteria used are given in Table 1. The numbers of both the types of bacteria were found by counting the two types of coloured colonies developing on the agar plates. Bacteria 4045 produced citron yellow colonies, but the spinel red bacteria (5654) sometimes produced white colonies as well. The red and the white colonies produced by the bacteria 5654 are of similar shape on agar plates. The number of active and cystic amoebae was counted only a few times during the course of this experiment by Cutler's method (1920).

Before selecting the species 4045 and 5654 preliminary tests were made with several species of soil bacteria to find out if there was antagonism between them: presence of species 4045 does not stop the growth of species 5654 and conversely.

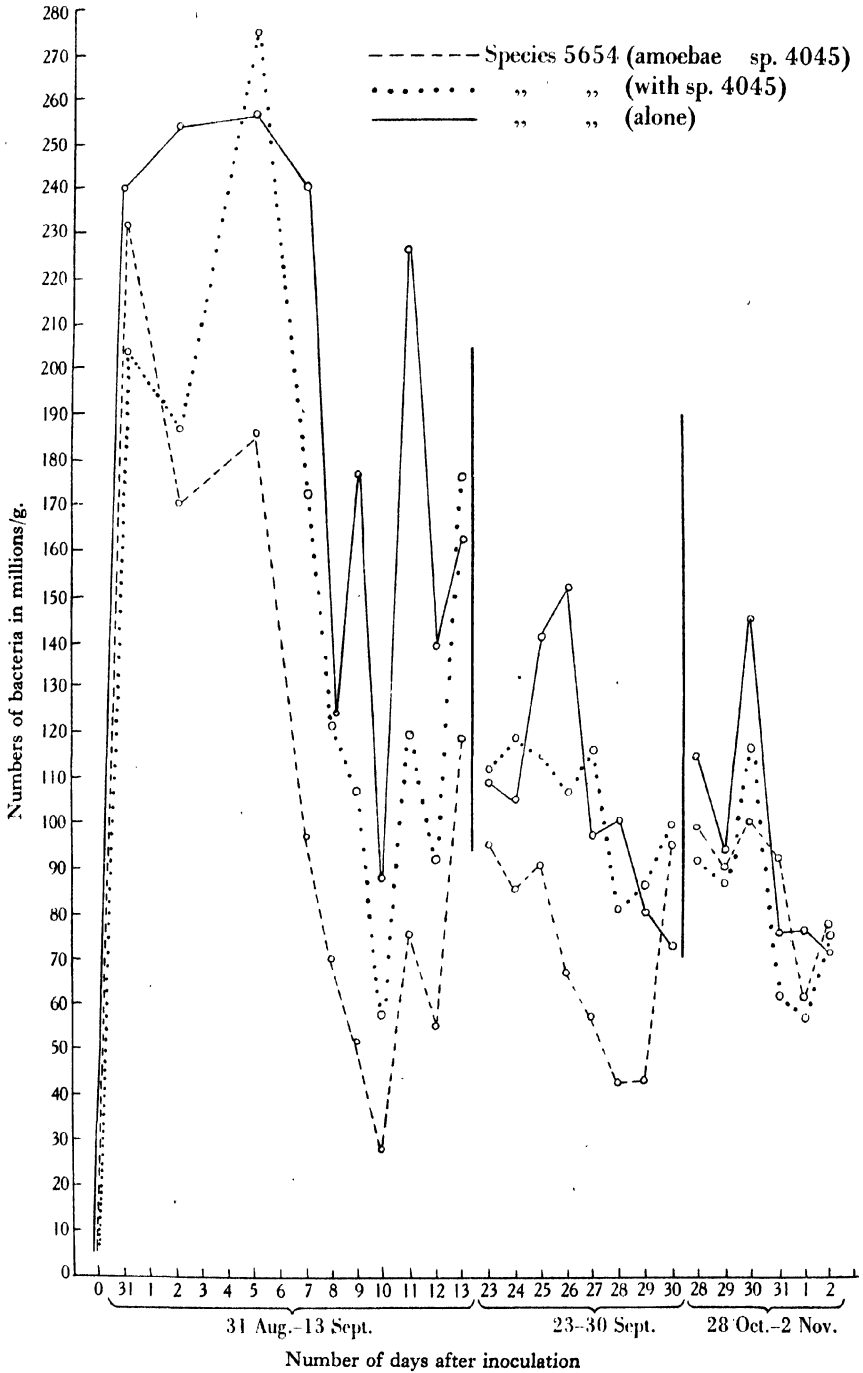
EXPERIMENTAL RESULTS

As pointed out in § A, bacteria 4045 is slowly eaten by the amoebae, and bacteria 5654 is not touched by them in pure culture. The present work was carried out to ascertain if similar results are obtained in sterilized soil. Results are shown in Table 2 and Text-figs. 2 and 3. In the case of bacterial species 4045 the presence of amoebae does not influence the result for the first week. The fluctuations in bacterial numbers, both in the soil containing the amoebae as well as the controls, are more or less similar. This can be explained by the fact that amoebae were inoculated mostly as cysts, and it takes some time before their active number is large enough to destroy the bacteria in sufficient numbers. A similar result was obtained by Cutler (1923). The results obtained from 9 to 13 Sept. show clearly that amoebae are definitely exerting their phagocytic influence on bacteria 4045. The



Text-fig. 2.

SELECTIVITY IN BACTERIAL FOOD BY SOIL AMOEBAE



Text-fig. 3.

count from 23 to 30 Sept. shows that, in the soil in which the amoebae were present, the number of bacteria 4045 is gradually falling and there is practically no sign of their increment or fluctuation. The bacterial numbers in control experiments are more or less stationary, with slight fluctuations (compare Cutler *et al.* 1922, and Thornton & Gray, 1934). The result obtained from 28 Oct. to 2 Nov. is more or less the same as that obtained from 23 to 30 Sept. This experiment clearly shows that amoebae destroy the bacteria 4045 in sterilized soil, the number of bacteria 4045 being at a much lower level in the soil containing amoebae than in the control soils (Table 2 and Text-fig. 2).

The results obtained in the case of bacteria 5654 are shown in Table 2 and Text-fig. 3. The counts from 7 to 12 Sept. show that the amoebae reduce the number of this bacteria. In pure culture bacteria 5654 is never eaten by the amoebae, but it seems that amoebae are able to eat bacteria 5654 up to the counting period of 30 Sept. The most interesting part of the result in the case of bacteria 5654 is shown by the bacterial counts from 28 Oct. to 2 Nov., during which period the amoebae are not able to eat the bacteria 5654. When the protozoan count was taken on 30 Oct. the amoebae were mostly found to be in the cystic condition (Table 3). During the period 28 Oct.–2 Nov., the number of bacteria 4045 is very low, and although the number of bacteria 5654 is high yet the amoebae are found in the cystic condition. It is reasonable to draw the conclusion that amoebae are unable to eat bacteria 5654 from 28 Oct. to 2 Nov. when the bacterial food 4045 was almost exhausted, and so they encyst. It is interesting to note that although there is plenty of food for the amoebae, mostly consisting of bacteria 5654, yet the amoebae do not eat that food, a result in complete agreement with that obtained in pure culture experiments.

The numbers of active and cystic amoebae were counted only three times during the experiment (Table 3) and it is clear that amoebae were present both in the active and cystic condition in the soil and that their number increased during the experiment. When the last count was taken, the majority were found to be in the cystic condition.

TABLE 3

Date	Amoebae (active + cystic)	Amoebae (cystic)	Amoebae (active)
12 Sept.	118,000	46,000	72,000
26 Sept.	220,000	118,000	102,000
30 Oct.	220,000	220,000	—

By comparing the results in control experiments B, C and D (Table 2) it is clear that the two types of bacteria (5654 and 4045) selected for the present work do not arrest the growth of each other. The fluctuations in their numbers in C and D are more or less the same as in B, in which there are both the types of bacteria 5654 and 4045.

DISCUSSION

It has been the experience of all previous workers that the main food of amoebae consists of bacteria either living or dead. Amoebae have also been cultivated on yeasts, and they may feed on fungal spores, algae, etc. Even, however, in the case of dead bacteria, killed either by heating or by any other way, there are only a few cases where amoebae have been cultured successfully. So far it has not been possible to cultivate amoebae on mainly liquid media without particulate food as is possible in the case of saprophytic or autotrophic

protozoa. Cutler, Crump and others believe that amoebae may be one of the factors keeping the number of bacteria in check in the soil, and Cutler *et al.* (1922) observed a definite inverse relationship between bacterial numbers and active amoebae in field soil. In view of the possibility that amoebae and probably also the flagellates lead a holozoic existence in the soil, where there is plenty of bacterial food supply, it is desirable to know whether amoebae eat all types of bacteria or whether they select among them, and in the latter case to find the reason for the selectivity.

Among the Gram-positive and Gram-negative bacteria tested amoebae showed no special preference for Gram-negative. This is contrary to the findings of Oehler (1916, 1924 *a, b*), but agrees with the results of Severtzova (1928) and Rice (1935). Bacterium $\lambda T 20$ is Gram-positive yet it is the best kind of food for the amoebae. Pigmentation in bacteria has no relation to their edible or non-edible quality. Similarly, in most cases, there is no evidence of the presence of endo- or exo-toxin in the non-edible bacteria which prevents them from being eaten by the amoebae, although the production of exo-toxin was observed in bacteria 2881 and may prevent it from being eaten by amoebae.

Among seventeen different kinds of bacteria, it has not been possible to discover any particular character which prevents them from being edible or non-edible for the amoebae. It may only be said that some bacteria are preferred by the amoebae to the others; e.g. some *Aerobacters* more than others. By developing a technique for carrying out feeding experiments with amoebae, it has been possible to show more clearly that amoebae are able to select their food, amoebae first eating the readily acceptable food and later the slowly acceptable food.

By inoculation experiments carried out in sterilized soil, it has been shown that amoebae are able to select their food, although the result is not exactly the same as in pure culture experiments, and the edible bacteria are not destroyed in large numbers. Severtzova (1928, p. 177) says: "Excepting one case the presence of amoebae in the soil did by no means affect the normal development of the bacteria in it. The amoebae did not hinder the development of the bacteria, even in those cases where the multiplying amoebae were placed under exceptionally favourable conditions. Concerning the question, whether amoebae can as sharply manifest their selective ability in the soil, as they do on artificial media, our preliminary experiments have given an affirmative answer in one point; in the presence of amoebae spore-bearing bacilli developed in the soil far more abundantly than did small, motile, non-spore-bearing bacteria, perhaps, because the latter were more readily attacked by the amoebae, though other causes may be responsible." In the absence of the experimental data, it is difficult to comment on the conclusions drawn by Severtzova. It must be pointed out that the amoebae are able to select edible from non-edible food in sterilized soil, although the numbers of bacteria destroyed by the amoebae in sterilized soil is very small compared with those in pure culture experiments. Further, Cutler (1923) showed that the presence of active amoebae in sterilized soil keeps the numbers of bacteria below the level that they would otherwise have attained.

It may be pointed out that species 4045, which has been selected in the present experiment, is not among those which are readily eaten by the amoebae in pure culture. It seems likely that, if instead of species 4045, a bacteria which is readily eaten by the amoebae had been selected it would have given much better results in selectivity experiments than has been obtained with bacteria 4045.

SUMMARY

1. A plate culture method, for carrying out work on the selection of bacteria for food by amoebae, is described.
2. It is shown that some species of *Aerobacter* are preferred by amoebae to others, though they are all morphologically and more or less physiologically identical.
3. Of a number of bacterial species, mostly rods of different sizes, some were completely rejected as food by the amoebae. Among those that were selected as edible, the amoebae showed varying degrees of preference. It has not been possible to determine why amoebae eat some kinds of bacteria rather than others, except possibly in the case of bacterium 2881.
4. Amoebae have no special preference for Gram-negative bacteria in comparison to the Gram-positive ones.
5. Amoebae are able to select edible from non-edible food whether the two types of food are offered side by side or on opposite sides of the plate culture.
6. It has been shown that the amoebae are able to select their food in sterilized soil, among edible and non-edible species of bacteria. In sterilized soil the amoebae do not destroy edible bacteria in large numbers as is seen in pure culture experiments.
7. The number of edible bacteria is very much reduced when amoebae are present. It seems very likely that the amoebae are not able to keep the numbers of non-edible bacteria in check as is the case with edible types of bacteria.

My best thanks are due to Mr D. Ward Cutler and Miss L. M. Crump for their never failing interest, suggestions and helpful criticisms throughout the course of this work, and for giving me the different species of bacteria from their personal collections.

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EXPLANATION OF PLATE 4

Fig. 1. Preference in the bacterial food, consisting of *Aerobacters*, by soil amoebae. 3 days old culture of amoebae.

Figs. 3, 4. Amoebae eat the edible and leave the non-edible kinds of bacteria when they are put side by side and touching each other. 4 days old culture of amoebae.

Figs. 2, 5 and 6. Amoebae select the edible from the non-edible food when several types of food are present. Fig. 5 is 5 days old, Fig. 6 is 8 days old, Fig. 2 is 6 days old culture of amoebae.

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THE INFLUENCE OF DIFFERENT BACTERIAL FOOD SUPPLIES
ON THE RATE OF REPRODUCTION IN *COLPODA STEINII*, AND
THE FACTORS INFLUENCING ENCYSTATION

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VARIOUS workers have claimed that factors such as drying of the culture medium, metabolic products of bacteria and Protozoa, age of the culture, hydrogen-ion concentration, lack of oxygen, physiological periodicity, lack or abundance of food, crowding of the individuals, temperature, etc. influence encystation in different Protozoa (see Penn, 1935, for bibliography). A knowledge of the causes of cyst formation might throw light on the role played by the Protozoa in soil economy, and especially on the cause of the daily fluctuations in the number of Protozoa recorded by Cutler *et al.* (1922). It was desired to find out whether the rate of reproduction is accelerated, in the case of *Colpoda*,¹ by the quality of the bacterial food or whether it is due to an "X" substance as suggested by Robertson (1921*a, b*, 1924*a, b, c*, 1927). In this connexion a strain of a nodule bacterium (310)² has been used: this strain produces growth-promoting substance for plants.

MATERIAL AND METHODS

Colpoda steinii, a common soil form, was obtained by diluting Barnfield soil (Rothamsted Experimental Station) in sterilized hay infusion of 1.5 % strength, and incubating at 19-20° C. for a few days. The animals used were originally derived from a single individual. Individuals were isolated with fine glass tubing and washed at least five times in sterile soil extract until free from other micro-organisms (see Parpart, 1928). The washed individuals were placed in hollow-ground slides in two or three drops of soil extract, and were supplied with a known species of bacteria as food. The slides were kept in Petri dishes as moist chambers and incubated for 2-3 days at a temperature of 19-20° C. till a large number of animals were obtained. Aseptic conditions were maintained throughout.

For counting the organisms haemocytometers with the Cropper ruling were used. The rate of reproduction for any time is calculated by the formula $(\log B - \log A) / \log 2$, where *B* is the number at the end and *A* the number in the beginning. Lugol was a satisfactory means of killing the animals and they did not burst after death. Sometimes there is considerable difference in the number of individuals counted when several consequent counts are taken. To reduce this error the animals in all the 625 squares of the Cropper ruling was counted, and the average of four such counts was taken as the number of individuals present at each counting period. The number of bacteria at each counting period was counted in a Thoma haemocytometer. The animals grow well in hay infusion, but after some time the solution becomes wholly unsuitable for the uniform suspension of the organisms. Soil extract was used throughout as a culture solution. The initial reaction of the medium was approximately pH 7.0.

¹ This genus was most probably used by Robertson in his later experiments, although he names his genus *Colpidium*. As far as the writer is aware the reproduction in *Colpidium* always takes place by binary fission as happens in *Paramecium* and other ciliates. It is only in *Colpoda* that the individuals round up and form cysts before they divide into four or eight individuals. Therefore, I think that Robertson used in his experiments *Colpoda* and not *Colpidium*.

² Given to me by Dr H. Nicol of the Bacteriology Department, to whom I wish to convey my sincere thanks.

OBSERVATIONS

(a) *The rate of reproduction in Colpoda steinii when fed with different bacteria*

Five types of bacteria were selected (1734, 07, R, S 21 and 310): species 1734 and 07 belong to the *Aerobacter* group and are more or less physiologically identical, R is a Radiobacter, 310 is a strain of nodule bacteria, and S 21 is a soil form (Table 1).

TABLE 1. *Characters of bacteria used as food*

Strain	Motility	Morphology	Liquefaction	Gelatine	Milk	Dextrose	Laevulose	Sucrose	Lactose	Dulcitol	Nitrate reduction	Indol	Locality	Colour	Gram-stain
07	o	Short rod	o	Thread	Acid curd R.L.	G 4.0	G 4.6	G <4.0	G <4.0	6.9	+++	++	Milk waste	Milky white	-
1734	o	Short rod	o	Thread	Acid curd R.L.	G 4.7	G 4.3	G 4.2	G 4.4	6.9	+++	Trace	Milk waste	Milky white	-
S 21	o	Very small rod	+	Cup	R.L.	6.5	6.3	7.1	6.9		+++	Trace	Barn-field F.Y.M.	Milky white	-
R (Acromobacter radiobacter)	+	Small rod	o			Acid	Acid	Acid	Neutral	Neutral	+++		Soil	Milky white	-

In a subculture made from a 48 hr. old parent culture, there is a lag period of shorter duration compared with the subcultures made from 72 or 96 hr. old parent. Cutler & Crump (1923*a, b*, 1924) found the same thing in the case of *Colpidium colpoda* and *Oicomonas termo*. In subcultures of 4 or 5 days old parent, the death rate is heavy during the first period of 24 hr., but the individuals that survive are able to reproduce more vigorously, and their reproductive rates, after the lag period, are generally higher than subcultures made from 48 hr. old parents. The lag period is due to the death of the individuals after inoculation into a fresh medium, and no resistant cysts have been seen to be formed during this period.

TABLE 2. *Effect of different numbers of Protozoa in subcultures on the rates of reproduction for the first 48 hr.*

Inoculum c.c.	No. of bacteria per c.c. (millions)	No. of <i>Colpoda</i> per c.c.	Ratio	No. of bacteria per c.c. after 48 hr. (millions)	No. of <i>Colpoda</i> per c.c. after 48 hr.	Ratio	Reproductive rate of 48 hr.
0.5	1200	4,000	300,000	960	39,200	24,489	3.29
1.0	1200	5,600	214,285	720	64,800	11,111	3.52
2.0	1660	12,400	133,871	860	31,200	27,589	1.33

In mass cultures the rate of reproduction is lower when the number of individuals in a subculture is increased considerably. Table 2 shows the reproductive rates in *Colpoda* for the first 48 hr. The amount of fluid, in which the subcultures were made, was the same in all

cases. In all the tables the bacterial numbers are expressed as a ratio, obtained by dividing the number of bacteria by that of the Protozoa.

Table 3 shows the reproductive rates in *Colpoda* for the first 24 hr. with bacterial food supply of 07, 1734, R and S 21. In all these experiments the amount of the inoculum and the culture fluid was nearly the same, and the cultures, from which the subcultures were made, were all of the same age (48 hr.). With bacterial food supply of 07 and 1734 there is very little difference in the reproductive rates of *Colpoda*. With species R the rate is lower than with 07 and 1734, although the number of Protozoa in the subculture is only 2800/c.c. compared with the numbers in 07 and 1734 (5600 individuals/c.c.). With species S 21 as food supply, the rate of reproduction in *Colpoda* for the first 24 hr. is 0.90. Here the initial number of individuals inoculated is greater than in the case of 07 and 1734, but there is a very marked difference in the reproductive rate in S 21 compared with 07 and 1734. It has been shown that amoebae prefer species S 21 to 07 and 1734, and species R is non-edible (Singh, 1941).

TABLE 3. *Reproductive rates in Colpoda steinii for the first 24 hr. with different bacterial food supply. Subcultures were made from 48 hr. old parents*

Bacteria	No. of bacteria per c.c. (millions)	No. of <i>Colpoda</i> per c.c.	Ratio	No. of bacteria per c.c. after 24 hr. (millions)	No. of <i>Colpoda</i> per c.c. after 24 hr.	Ratio	Repro- ductive rate of 24 hr.
07	1680	5600	300,000	820	58,800	13,946	3.39
1734	1440	5600	257,142	1000	41,200	24,271	2.87
R	1660	2800	578,573	1700	10,400	163,461	1.89
S 21	1520	6400	227,500	1360	12,000	113,333	0.90

In the strain of nodule bacteria (310) *Colpoda* does not reproduce, and even if it divides the reproduction is very slow. Large numbers of species 310 were inoculated into 10 c.c. of soil extract. Later the Protozoa from a 48 hr. old culture, maintained with species S 21, were inoculated. There was very little food in the culture from which the inoculum was made. The initial number of Protozoa in the subculture was 1200/c.c.; after 24 hr. the number fell to 600/c.c., and after another 24 hr. the number of Protozoa was decreased so much that it was not possible to count them by the method used. There were many resistant cysts present in the culture and the individuals were very small. On the third day the number of Protozoa increased to 6000/c.c., and some of the individuals were big; some dividing cysts were also present. After another 2 days the number fell to 1200/c.c., although species 310 was present in quantity. The individuals were small, and resistant cysts were numerous. Such a result has been obtained several times when a subculture was made in which the food supply was species 310.

The experiment suggests that the Protozoa eat species 310 little if at all. The number of *Colpoda* increases with the increase of the contaminating species S 21, and in its absence the Protozoa become progressively smaller and finally encyst.

(b) *The effect of bacterial and protozoal products on the rates of reproduction*

Exp. 1. Into a small flask containing soil extract, species S 21 and *Colpoda* were inoculated, and were incubated at a temperature of 19–20° C. After a few days, when the *Colpoda* had eaten nearly all the bacteria, more of the same species were supplied and the flask was incubated for a period of

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7-10 days. Then the soil extract was filtered and the filtrate was divided into two small flasks each containing 9.5 c.c. The soil extract of one flask was heated to 70-80° C. for 1 hr. and then cooled. In a third flask 9.5 c.c. of sterile soil extract was taken as control. The three flasks containing the same amount of liquid were first inoculated with S 21 and then with approximately equal numbers of *Colpoda* from a 48 hr. old culture, which had been growing on the same bacterial food supply. The rates of reproduction in the three cases are given in Table 4.

TABLE 4. *Reproductive rates of the first 48 hr. in Colpoda steinii in the presence and absence of the bacterial and protozoal metabolic products*

Bacteria	No. of bacteria per c.c. (millions)	No. of <i>Colpoda</i> per c.c.	Ratio	No. of bacteria per c.c. after 48 hr. (millions)	No. of <i>Colpoda</i> per c.c. after 48 hr.	Ratio	Reproductive rate of 48 hr.
S 21 (Control)	1360	1200	1,333,333	660	47,600	13,865	5.30
S 21 (Filtrate)	1180	1000	1,180,000	820	13,600	59,944	3.76
S 21 (Filtrate heated for 1 hr. at 70-80° C.)	1000	800	1,250,000	680	37,600	18,085	5.55

It is clear that the rates of reproduction in the control and in the filtrate, which was heated to 70-80° C. for 1 hr., are the same. The rate of reproduction in the filtrate, which was not heated, is slightly lower than in the other two cases. It would seem that either the bacteria or the Protozoa, or both, produce some toxic substance which is thermolabile at a temperature of 70-80° C.

Exp. 2. The bacteria (310) were inoculated into sterile soil extract and the liquid was incubated at 25° C. for 10 days. The soil extract was filtered at the end of this period. The experiment was arranged in the same way as the previous one. The results obtained are shown in Table 5.

TABLE 5. *Effect of the presence of the filtrate of nodule bacteria (310) and its absence on the rate of reproduction in Colpoda steinii*

Bacteria	No. of bacteria per c.c. (millions)	No. of <i>Colpoda</i> per c.c.	Ratio	No. of bacteria per c.c. after 24 hr. (millions)	No. of <i>Colpoda</i> per c.c. after 24 hr.	Ratio	Reproductive rate of the first 24 hr.
S 21 (Control)	1720	4000	430,000	1380	22,400	61,607	2.48
S 21 (Filtrate of nodule bacteria)	1740	3600	483,333	1540	12,400	124,193	1.78
S 21 (Filtrate of nodule bacteria heated for 1 hr. at 70-80° C.)	1900	3600	527,777	1540	19,200	80,308	2.41

The product of the nodule bacteria, which is of the nature of a growth-promoting substance for plants, has no effect on the rate of reproduction in *Colpoda*. The unheated filtrate of the nodule bacteria seems to depress very slightly the reproductive rate of *Colpoda* during the first 24 hr.

(c) *The rate of reproduction in isolated individuals*

Single individuals washed several times in sterile soil extract were transferred to two drops of soil extract in hollow-ground slides and supplied with approximately the same amount of species S 21 as food supply. The slides were put into moist chambers. Similarly two and four individuals were

isolated, washed and were put into two drops of soil extract and supplied with approximately the same amount of species S 21 as food supply. The chambers were incubated at a temperature of 19–20° C. for 24 hr., and at the end of this period the animals were killed by lugol solution. The number of individuals present was counted and their rates of reproduction for the first 24 hr. calculated. All the animals that were isolated came from cultures of the same age, maintained on species S 21 as food supply.

The average reproductive rates show no evidence of allelocatalysis, and the rates of reproduction are practically the same whether one, two or four individuals are isolated in the same amount of culture medium containing nearly the same amount of food supply (Table 6).

TABLE 6. *Rate of reproduction during the first 24 hr. in one, two and four individuals*

	1 animal	2 animals	4 animals
Average reproductive rate during the first 24 hr.	3.71	3.67	3.61
Number of cases	8	8	8

As already mentioned, in mass cultures, the rates of reproduction fall when the amount of inoculum is increased. The results obtained in mass cultures are more reliable than when one, two or four individuals are isolated. In isolating and washing individuals it is possible that injuries may be caused to the cells, which may not only stop their reproduction but may induce death after some time. The death of weak and unfit individuals in mass cultures by the shock of being transferred to a fresh medium or by some other cause would not affect the results to such an extent as it would do in the case of one, two or four isolated individuals.

(d) Encystation

Of the two types of cysts in *Colpoda* the "reproductive cyst" is several times larger than the resistant or "dauer cyst". From each reproductive cyst two, four or eight individuals emerge by a rupture in the wall. I have generally observed four individuals emerging from a single reproductive cyst. In *Colpoda*, crowding, hydrogen ion concentration, excretion products, and lack of food have been claimed by various workers to be the inducers of encystment (Barker & Taylor, 1931; Taylor & Strickland, 1938). The present work mainly deals with the food factor and the excretion products, though other factors have also been taken into account.

In experiments to test the nutritive values of different species of bacteria it was noted, in every case, that the resistant cysts are formed when the bacterial food supply was almost or completely exhausted. The resistant cysts are never formed when the individuals are large. The time taken by the individuals to encyst depends upon the amount of undigested food present in the body of the animals.

An experiment was set up in which food was added over a period of 9 days, whenever there was scarcity of food in the culture. Into 10 c.c. of soil extract large numbers of bacteria S 21 were inoculated, and then the Protozoa from a 72 hr. old culture. After the first maximal number of Protozoa (81,600/c.c.) was reached the individuals began to encyst, and at this stage no reproductive cysts were present, and all the individuals were very much smaller than those found in a well-fed culture. Plenty of species S 21 was supplied when the number of Protozoa was 51,200/c.c., at which stage only very few resistant cysts were present in the culture. After the addition of food the number of Protozoa per c.c. decreased for a time, this being due to the death of a large number of individuals and not to cyst formation.

A few hours after the supply of food the size of the individuals began to increase and a few reproductive cysts were present in the culture. Later all the individuals were large, and numerous reproductive cysts were present. The number of Protozoa rose to 312,000/c.c. and there were practically no small individuals and no resistant cysts in the culture medium. As the food supply was nearly finished, several loopfuls of bacteria were added. This led to a sudden fall in the protozoal number, shown clearly by the subsequent counts. Although the number of Protozoa fell from 312,000/c.c. to 31,600/c.c. no resistant cysts were formed in the culture. This was due to death of individuals, which in aged or nearly starved cultures of 5-10 days old has been repeatedly observed after heavy supply of bacterial food. If counting had been continued there would have been further increase in the protozoal number, as was observed before, but as little soil extract remained the experiment was discontinued.

Sometimes practically all the Protozoa die in old cultures when several loopfuls of bacterial food are added, and when the individuals are small and no reproductive cysts are present. This phenomenon has been observed in *Colpoda dudenaria* by Taylor & Strickland (1938), who state: "The lethal effect of a dense concentration of these bacteria on *Colpoda dudenaria* is due primarily to the lack of oxygen, and that under the conditions of this experiment the metabolites neither prevent division nor induce encystment."

In previous experiments, it was shown that Protozoa and bacteria produce a thermolabile substance which has a slight effect on the rate of reproduction. It may be possible that the accumulation of large amounts of toxic products may have caused the death of numerous weak and unfit individuals which had become very small owing to the lack of food in the previous experiment. It has been observed that in an old and well-fed culture the death-rate of the individuals after heavy inoculation is not so great as in starved cultures. As pointed out by Taylor & Strickland (1938) it may be possible that the lack of oxygen may also be responsible for the death of the individuals. I have never observed the formation of resistant cysts in the presence of toxic substances produced by bacteria and Protozoa.

In the presence of unfavourable bacterial food permanent cysts may be formed. *Colpoda* is unable to grow when nodule bacterium 310 is supplied as food and when starved individuals, which have become small, are inoculated into soil extract containing bacterial species 310 resistant cysts are formed within a few hours. If well-fed animals are inoculated, they become smaller and smaller and finally encyst. Some of these Protozoa excyst but they again encyst, on account of the lack of favourable food.

In *Colpoda* temperatures up to 25° C. have no effect in inducing cyst formation in the presence of food. No relationship between the age of the culture and cyst formation could be noted, and it does not seem, in the present case, that physiological periodicity, crowding, the age of the culture and the abundance of food are the causes of cyst formation. When a culture containing abundant food is dried the individuals die instead of forming resistant cysts. It was shown by Taylor & Strickland (1938) that growth and encystment occurs in *Colpoda dudenaria* as readily at a pH value of 8.2 as that of pH 6.0.

(e) Excystation

In liquid medium (soil extract, hay infusion, etc.) freshly formed resistant cysts excyst both in the presence and absence of food supply. The mechanism by which a single individual emerges from the resistant cyst is the same as described by Goodey (1913). After their

emergence, when there is sufficient food supply, the individuals increase in size and form reproductive cysts: in the absence of food supply the individuals encyst again within a few hours. A large proportion of resistant cysts do not excyst even if there is sufficient amount of food supply. Hollow-ground slides containing resistant cysts were incubated both at 20 and 25° C.: some were supplied with bacterial food and the others were not. In such experiments excystation occurred both in the presence and absence of food only in the case of recently formed cysts. More cysts excysted when they were kept at 25° C. than when they were kept at 20° C.

The resistant cysts, which had been formed in hollow-ground slides when the food supply was finished, were dried by allowing the soil extract to evaporate at a temperature of 25° C. They were then kept in these hollow-ground slides for more than 3 months at a temperature of 25° C. At the end of this period the cysts were moistened with a few drops of soil extract and in addition some were supplied with bacteria S 21. The cysts were incubated at 25° C. in a moist chamber. In 12–24 hr. many cysts containing species S 21 excysted and later the individuals increased in size and reproduction took place by the formation of reproductive cysts. In the presence of soil extract excystation did not occur up to 7 days. At the end of this period, when many contaminating bacteria had developed, some of the resting cysts excysted. It has been reported by Barker & Taylor (1933), Thimann & Barker (1934) and Taylor & Strickland (1935) that extracts of vegetable or animal tissues induce excystment of *Colpoda*. This experiment shows that the bacteria produce some substance which induces some of the dried cysts, of more than 3 months old, to excyst. It may be possible that many of these resistant cysts after a long period, in the dried condition, were dead, and so were incapable of excystation. Goodey (1913) found that if *Colpoda* cysts were kept in dry condition for a few weeks their power of excystation was rapidly diminished, but in the recently formed cysts excystation was most rapid. The excystation took place in distilled water, tap water, hay infusion and in soil extract, but was inhibited in an acid medium. Rumbler (1888) could not keep dried cysts of *Colpoda* more than 3 weeks. Many resistant cysts, whether they are freshly formed or dried for a long time, do not excyst under the conditions of the present experiment. When the cysts are left in the dried condition in hollow-ground slides for more than a year, they are incapable of excystation and all of them die.

DISCUSSION

The literature on allelocatalysis and the effect of different bacteria on the growth and reproductive rates of Protozoa has been summarized in two recent reviews by Luck *et al.* (1931) and Hammond (1938). It is enough to point out that there is no allelocatalysis in *Colpoda*, and that different types of bacteria influence the growth and the reproductive rates in Protozoa.

When bacterized hay infusion is used as food for the ciliates, they may encyst when the favourable bacterial food supply is finished, even if the amount of the unfavourable food increases to a large extent. It is possible that some of the results obtained on encystation in ciliates, in the presence of food, may be due to this reason. Such a result has been obtained in *Colpoda* in the presence of a strain of nodule bacteria. If one is studying the food factor in connexion with encystation in ciliates and other Protozoa it is of the utmost importance to work under sterile conditions and to use only one type of favourable bacteria as food.

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Food is not the only factor which induces cyst formation in Protozoa. The writer has observed that in a liquid medium, soil amoebae encyst even in the presence of favourable food supply. In this case temperature, metabolic products, etc., seem also to influence the induction of cyst formation. In different ciliates other factors, apart from the food, may influence cyst formation, but many of the controversial results which have been obtained are probably due to the fact that the experiments were not carried out under sterile conditions, and that a favourable bacterial food supply was not used.

SUMMARY

1. The rate of reproduction in *Colpoda* varies considerably with the bacterial food supply, the age of the culture, the size of inoculum, and the metabolic products.
2. The metabolic products of bacteria and Protozoa have a slight retarding effect on the rate of reproduction. There is no evidence of allelocatalysis in *Colpoda* either in isolated individuals or in mass cultures. The evidence obtained in mass cultures shows that the rate of reproduction in a subculture containing a smaller number of Protozoa is better than when the number is considerably increased.
3. The resistant or "dauer" cysts are formed only when there is practically no bacterial food present in the culture solution or in the presence of unfavourable food supply, e.g. strain of nodule bacteria 310. The metabolic products of bacteria and Protozoa and certain other factors have no influence on the formation of resistant cysts in *Colpoda*.
4. Encystation takes place even in dried cysts of more than 3 months in the presence of bacteria. No excystation takes place in such dried cysts when they are moistened with only soil extract without the presence of bacteria. Thus it seems that bacteria produce some substance which induces excystation in the case of *Colpoda*.

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AN ANALYSIS OF FOUR YEARS CAPTURES OF INSECTS IN A LIGHT TRAP. PART II.¹ THE EFFECT OF WEATHER CONDITIONS ON INSECT ACTIVITY; AND THE ESTIMATION AND FORECASTING OF CHANGES IN THE INSECT POPULATION

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(Read 7th February, 1940.)

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INTRODUCTORY AND OUTLINE OF ARGUMENT.

A LIGHT trap, with an electric light of approximately 300 candle-power at a height of about 3 feet 6 inches above the ground, was exposed in one of the fields at Rothamsted Experimental Station, Harpenden, about 25 miles north of London, on a total of 1407 nights between the 1st March 1933 and the 28th February 1937. The catch each night was counted and sorted into orders, and some of the orders again sorted to families and species. The total catches in the four successive years were 109,344; 103,362; 399,006 and 242,822 insects respectively. The number caught per night varied from zero on many nights in the winter months to a maximum of approximately 73,000 on one night at the end of June 1935. There were, however, only 13 occasions when the catch was over 10,000 insects and these were all in 1935 and 1936.

A full account of the trap has already been published (Williams 1935), and in the first half of this present paper (Williams 1939) the results have been discussed in their bearings on the distribution of the catch among the different orders of insects, the sex proportions, phenology and the time of flight during the night.

The present section is an attempt to analyse the results in so far as they throw light on the problem of the influence of weather conditions on the catches; and hence, if one assumes the catches to be a representative sample, on the activity and on the basic population of the insects.

As this analysis is rather long and complicated, and as it contains references to methods that were found unsuccessful as well as to those that were successful, it has been thought desirable to start with an outline of the method and results in order that the threads can be more easily held together later.

The argument developed is as follows: The number of insects caught in the trap on any one night is mainly determined by two factors; (1) the activity of the insects, and (2) the total population available for sampling.

The activity varies rapidly from hour to hour and from night to night and is determined largely by the weather conditions of the moment, and (in this locality) particularly by temperature and wind and to a less extent by humidity and other factors.

The population changes happen more slowly and are determined more by the weather conditions some time previous than by those at the moment; except perhaps in the case of the sudden emergence of a large number of insects from the pupal stage when the weather becomes warm after a cold spell.

Thus, if one considers the difference in catch between two successive nights,

the effect will be largely due to activity; whilst the difference in catch between two similar nights in different months will be largely due to population. The difference in average catch between June of one year and June of another will be still more dependent on population differences.

By the statistical method of "partial regressions" and by using as data the difference between successive days, values have been obtained which it is believed show very closely the "activity" effect on the catch due to unit changes in the maximum temperature of the previous day, the minimum temperature of the night, the wind force during the night, etc. These have been expressed as percentage changes in catch. Thus it will be shown that 1° F. increase in minimum temperature of the night adds on an average 18% to the catch; 1° F. increase in maximum temperature adds 5% to the catch, and one group increase in wind (see later for explanation) reduces the catch by 28%.

These values can be used to correct the mean catch in any one month for the departures of that month's weather conditions from the normal. A new figure is thus obtained for what the mean catch for that month would have been if weather conditions had all been normal. If these "mean catch values under normal conditions" are calculated for the same month in a series of years the differences between them (assuming that errors are as likely in one direction as another) are measures of the population differences between the months.

In this way a measure of the percentage or logarithmic departure of the population from the normal has been obtained from the trap data over a series of 48 months. The method of partial regressions can now be applied to these figures to show to what extent they can be accounted for by changes in the weather conditions in the preceding months. The factors taken for this were minimum temperature and rainfall in the three months previous; and two series of regressions have been obtained: one for summer (May to October) and one for winter (November to April). The figures show that in winter, temperature is the most important factor and rainfall can be practically neglected; while in summer rainfall is the most important factor and temperature changes can be neglected.

From the regressions thus obtained a forecast of the probable population changes can be made from a knowledge of the weather conditions in the three months previous to the forecast, without using the trap figures.

When these estimations are made and compared with the population changes calculated from the trap catch it is found that 50-60% of the variance of the latter can be accounted for; and undoubtedly a higher figure could be obtained if data were available to calculate separate regression values for spring, summer, autumn and winter.

The method has so far only been applied to the total insect catch (that is to say, to the sample thus obtained of the total positively phototropic night population). New data are being collected and the analysis continued to see to what extent the methods can be applied to single species; and how the difficulties, arising from the rapid rise and fall of population during a brood, can be overcome. An attempt has been made in this paper to explain graphically some of the mathematical processes used, and to develop them from simple examples to more complicated ones, so that the main principles of both method and results can be understood by any entomologist with some knowledge of mathematics. The work will lose half its value if the mathematical side is only understood by statisticians with no knowledge of the idiosyncrasies of insects, and the entomological side only by entomologists who fail to understand that mathematics is only a condensed form of reasoning.

METEOROLOGICAL RECORDS AVAILABLE.

Rothamsted Experimental Station ranks as a First-Class Meteorological Station and the majority of the records are taken within 240 yards of the trap itself.

Records are available on continuously recording instruments of barometric pressure, air screen temperature, rainfall, wind (direction and force), total radiation, bright sunshine and (for most of the years) relative humidity. Records are taken once or twice a day of many other factors, including air screen temperature, soil temperature at various depths, and wet- and dry-bulb temperatures. Maximum and minimum temperatures are recorded daily in the screen, and also the minimum on the ground (grass minimum) and the maximum of the black bulb in the sun. Notes are also taken each day of the prevalence of dew, fog, snow, frost, etc. etc.

In addition to the above it was found necessary for the present investigation to add instruments for measuring the duration of bright moonlight (see Williams and Emery 1935) and the duration of night cloud. The instrument for the latter was adapted from one already in use at Greenwich Observatory (see Anon. 1931 and Williams 1936b).

There was, therefore, available for comparison with the trap captures an excellent series of measurements of the physical environment; and, as many of the records have been taken for over fifty years, good average values were available as a measure of normal conditions.

All temperatures are given as ° F. unless otherwise stated.

Normal climatic conditions at Rothamsted.

Table 1 shows the average values of some of the principal climatic factors for the station.

TABLE 1.

Mean weather conditions at Rothamsted over 39-79 years and figures showing how the conditions in the four trap years differed from the long-period means.

Long period means.														
	Temperature						Daily range	Sunshine	Rain		Rainy days			
	Max.		Min.		Mean				In. per month					
	50 yrs.	4 yrs.	50 yrs.	4 yrs.	50 yrs.	4 yrs.	50 yrs.	4 yrs.	39 yrs.	4 yrs.	79 yrs.	4 yrs.	79 yrs.	4 yrs.
March . . .	48.2	+ 1.7	34.1	+ 2.0	41.1	+ 1.9	14.1	- 0.3	3.74	+ 0.65	1.992	- 0.286	14	± 0
April . . .	53.6	- 1.4	37.3	+ 1.1	45.5	- 0.1	16.4	- 2.4	5.22	- 0.82	1.985	- 0.228	13	- 0.5
May . . .	60.9	- 0.6	44.1	- 0.9	52.5	- 0.7	16.8	+ 0.4	6.60	- 0.63	2.152	- 1.021	13	- 3
June . . .	66.1	+ 0.9	48.4	+ 1.9	57.2	+ 1.5	17.7	- 1.0	6.81	- 0.12	2.243	+ 0.655	12	+ 3
July . . .	69.4	+ 2.3	52.0	+ 1.8	60.7	+ 2.0	17.4	+ 0.6	6.50	+ 0.94	2.640	- 0.664	13	- 3
Aug. . .	68.4	+ 2.4	51.6	+ 0.8	59.9	+ 1.7	16.7	+ 1.7	6.05	+ 0.47	2.659	- 1.625	14	- 5.5
Sept. . .	63.8	+ 1.3	47.9	+ 2.7	55.8	+ 2.0	15.9	- 1.4	5.10	- 0.18	2.391	+ 0.715	13	+ 1
Oct. . .	55.6	- 0.6	42.1	+ 1.0	48.9	+ 0.2	13.5	- 1.5	3.50	- 0.36	3.059	- 1.155	17	- 1
Nov. . .	47.9	- 1.3	36.6	+ 0.4	42.3	- 0.5	11.3	- 1.7	2.17	- 0.46	2.701	+ 0.220	16	- 2
Dec. . .	43.8	- 1.0	33.8	+ 0.8	38.8	- 0.1	10.0	- 1.8	1.41	- 0.05	2.637	- 0.089	17	- 0.5
Jan. . .	42.8	+ 0.4	32.6	+ 1.5	37.7	+ 1.0	10.2	- 1.1	1.68	- 0.08	2.427	+ 0.304	17	+ 1
Feb. . .	43.8	± 0	32.6	+ 1.0	38.1	+ 0.5	11.2	- 1.2	2.46	+ 0.14	1.910	+ 0.338	14	+ 1
Year . . .	—		—		—		—		1661 hrs.		28.72 ins.		173	

It will be seen that, during the past 50 years, July has the highest average maximum temperature (69.4° F.) and January the lowest (42.8° F.). July has also the highest minimum (52° F.) and February the lowest (32.5° F.). July and January have respectively the highest and lowest mean temperatures (60.7 and 37.7° F.); the annual range of the mean temperature is therefore only 23° F.

The sunshine has averaged 1561 hours per year (39 years). June has the highest, 6.81 hours per day and December the lowest, 1.41 hours per day.

The rainfall is low and is evenly distributed throughout the year. The average (79 years) is 28.7 inches and no month averages less than 1.9 or over 3.1 inches. The number of rainy days (*i.e.* days with 0.01 inch or over per day) is also very even throughout the year. There is, as is usual in England, no definite wet or dry season.

Weather in the four trap years.

As it is only possible to compare the catch in each of the four trap years with the mean catch during the four years it has been considered necessary that any comparisons made in weather conditions should be with a similar mean.

Table 1 therefore shows how the mean of the four years in which the trap was used differed from the long-range means; and Table 2 shows how each of the four years differed from their own mean.

An examination of Table 1 shows that the 4 years average minimum temperatures were above the 50 years normal in all months except May, and that the maximum temperatures were above the average in January and March and in June to September. Sunshine was only above the average in February and March and in July and August. Rainfall was in general below the average.

An examination of Table 2 shows that the successive years differed as follows from the four year average.

TABLE 2.

Departures of the chief weather factors in the four trap years from the average of these four years.

Temperature	Max.				Min.				Mean				Daily range			
	33	34	35	36	33	34	35	36	33	34	35	36	33	34	35	36
March	+3.3	-2.8	-0.5	-0.1	+0.1	-2.5	±0	+2.2	+1.7	-2.7	-0.2	+1.0	+3.2	-0.3	-0.5	-2.3
April	+3.0	+1.2	-0.5	-3.8	+1.1	+0.2	+0.6	-2.0	+2.0	+0.7	±0	-2.9	+1.9	+1.0	-1.1	-1.8
May	+1.1	+0.8	-2.6	+0.8	+2.0	-0.4	-2.0	+0.2	+1.5	+0.1	-2.3	+0.5	-1.0	+1.1	-0.7	+0.5
June	+1.4	+0.4	-0.8	-1.0	-0.6	-0.5	+0.8	+0.4	+0.4	-0.1	-0.1	-0.4	+2.0	+0.9	-1.6	-1.4
July	+1.8	+3.3	+1.1	-6.2	+1.5	-0.5	-0.4	-0.8	+1.7	+1.4	+0.4	-3.4	+0.2	+3.7	+1.4	-5.5
Aug.	+3.7	-2.3	+1.0	-2.3	+1.7	-1.5	±0	-0.2	+2.7	-1.9	+0.5	-1.3	+2.0	-0.8	+1.0	-2.1
Sept.	+2.2	+1.7	-1.6	-2.4	+0.8	-0.8	-1.3	+1.4	+1.5	+0.5	-1.4	-0.5	+1.4	+2.5	-0.3	-3.8
Oct.	+0.3	+1.2	-0.6	-1.0	+1.0	+1.5	-0.8	-1.7	+0.6	+1.4	-0.7	-1.4	-0.7	-0.3	+0.2	+0.7
Nov.	-1.5	-0.2	+1.9	-0.2	+0.6	+0.8	+1.0	-2.3	-0.5	+0.3	+1.4	-1.3	-2.1	-1.0	+0.9	+2.1
Dec.	-6.4	+6.3	-1.9	+2.0	-5.2	+7.3	-1.9	±0	-5.8	+6.8	-2.0	+1.0	-1.2	-1.0	+0.2	+2.0
Jan.	-0.1	-0.5	-0.7	+1.4	-2.0	+1.4	-0.5	+1.1	-1.1	+0.4	-0.6	+1.2	+1.9	-1.9	-0.2	+0.3
Feb.	-1.0	+2.5	-4.1	+2.6	-3.0	+3.0	-3.0	+3.1	-1.9	+1.5	-3.5	+3.9	+1.3	-0.2	-0.8	-0.2

	Sunshine (hrs. per day)				Rainfall (in. per month)				Rainy days			
	33	34	35	36	33	34	35	36	33	34	35	36
March	+1.96	-0.29	-0.06	-1.62	+0.98	+0.63	-1.16	-0.46	+2	+2	-4	0
April	+0.71	-0.37	-0.18	-0.17	-0.89	-0.40	+1.82	-0.53	-5.5	+0.5	+6.5	-0.5
May	-0.54	+0.51	+0.28	-0.26	+0.36	-0.30	+0.62	-0.69	+5	-1	-	-3
June	+1.33	-0.53	-0.19	-0.60	-1.90	-1.23	-0.08	+3.20	-2	-4	+2	+4
July	+0.50	+1.42	+1.60	-3.54	-0.63	-0.91	-1.06	+2.60	-1	-2	-5	+8
Aug.	+1.32	-0.70	+0.06	-6.67	-0.47	+0.74	+0.47	-0.74	-1.5	+4.5	+0.5	-3.5
Sept.	+1.19	+0.83	+0.08	-2.11	-0.84	-0.46	+1.07	+0.22	-4	-3	+2	+4
Oct.	-0.09	-0.40	+0.48	-0.01	-0.57	-0.02	+0.87	-0.27	-2	-2	+4	-1
Nov.	±0	-0.18	+0.35	-0.17	-1.63	-1.17	+2.09	+0.71	-2	-5	+7	0
Dec.	-0.03	-0.69	+0.17	+0.56	-2.11	+2.28	+0.50	-0.47	-3.5	+8.5	+0.5	+0.5
Jan.	+0.24	-0.09	±0	-0.17	-0.79	-1.90	+1.14	+1.55	+1	-8	+1	+5
Feb.	+0.83	-0.71	+0.19	-0.30	-1.80	+0.41	-0.12	+1.61	-9	+4	0	+6

1933-34. *Spring* (March to May). Maximum, minimum and mean temperatures all above average.

Summer (June to August). Maximum and mean temperatures all above average; minimum above average in July and August. Sunshine average. Rainfall below in all months.

- Autumn* (September to November). Minimum temperatures all above average. Maximum above in September and October. Rainfall all below average.
- Winter* (December to February). Maximum and minimum temperatures and rainfall all below average.
- General Characteristics.* Temperatures above average except in winter, rainfall much below average except in spring.
- 1934-35. *Spring.* Minimum temperatures average or below; rainfall above in March, below in April and May.
- Summer.* Minimum temperatures below average; rainfall below in June and July.
- Autumn.* Mean temperatures average; rainfall below.
- Winter.* Minimum temperatures above average especially in December, which had the highest value in 50 years. Maximum temperatures average or above, sunshine below average. Rain above in December, below in January.
- General Characteristics.* Minimum temperatures above average October to February, below in March and May to September. Sunshine generally below average. Rainfall below until the winter when the long drought ended.
- 1935-36. *Spring.* Maximum temperatures below average; rainfall above in April and May, very cold spell in mid-May.
- Summer.* Maximum and mean temperatures and sunshine above normal in July and August; rainfall below in May and June.
- Autumn.* Temperatures below average in September and October, above in November. Rain and sun above normal.
- Winter.* Temperatures all below average. Sunshine average or above. Rainfall above in December and January.
- General Characteristics.* Temperatures rather below average; rainfall above, and previous drought conditions replaced in some places by floods.
- 1936-37. *Spring.* Temperatures irregular. Sun and rain below average.
- Summer.* Temperatures mostly below average. Sun below, rain very high in June and July; 3.088 inches on 21st June.
- Autumn.* Temperatures and sunshine below average; rainfall somewhat above.
- Winter.* Temperatures above average; rainfall much above in January and February.
- General Characteristics.* Generally cool and rain above average.

The highest maximum temperature recorded during the four years was 89.3 on the 27th July 1933 and the lowest maximum 28.3 on 27th January 1937. The highest screen minimum was 65.3 on 3rd August 1933 and the lowest 13.4 on the 23rd December 1935. The total range of air temperature experienced was therefore 75.9° F. (42.2° C.).

The lowest grass minimum was 9.6° F. on the 12th February 1936.

NOTES ON THE STATISTICAL ANALYSIS OF THE RESULTS.

Sources of error.

Efficiency of the trap.

The trap, like all others, is by no means perfect. Some insects may fly round and fail to enter. More rarely some may escape after having entered.

Inefficiencies of this kind, provided that they are constant, should not affect the analysis of the results. The more serious errors are those which alter from time to time. For example, the intensity of the light produced varies approximately as the cube of the voltage. In the first year of use the trap was at the end of a field line over a quarter of a mile long on a 100-volts transformer circuit which was at times much overloaded; as a result the voltage was usually between 85 and 90 instead of 100. This difficulty was overcome by the use of "barretter" stabilising valves (see Williams 1935 : 526). At the end of the first year the current was changed to a main circuit at 240 volts and variations were so much reduced that the barretters were unnecessary.

The candle-power of a lamp also decreases as the lamp is used and is usually 10–15% less after the lamp has been in use for 1000 hours. This deterioration is more rapid if the voltage of the circuit is higher than the standard for the lamp and much slower if the voltage is lower. To reduce the error from this source as far as possible a lamp of the same voltage as the circuit was used, but a resistance of about 700 ohms was placed in parallel with the lamp so that the current going through the lamp was reduced. This lowered its candle-power but greatly increased its length of life at that candle-power.

Any likelihood of insects entering the trap and then escaping can only be reduced by keeping the killing-bottles in good condition. This has been done by renewing them whenever there was any sign of failure.

When using the trap to find the time of flight at night of various insects,

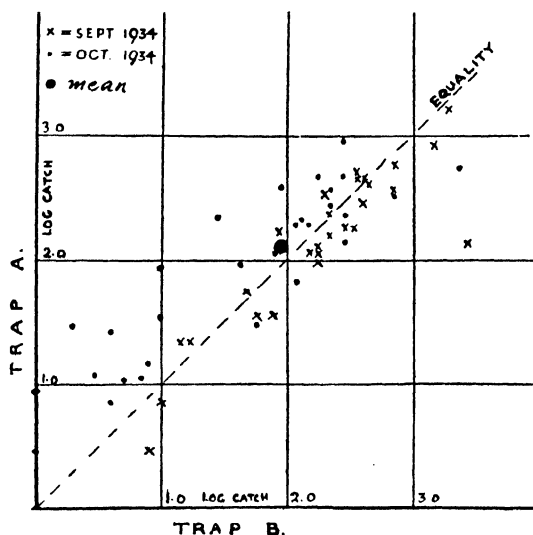


FIG. 1.—Scatter diagram showing relation between simultaneous catches in two light traps during September and October 1934. All insects.

any delay in the insect entering the trap or being killed when it enters may cause all the results to be too late. The trap was under close observation from this point of view on many occasions and it was found that there was seldom a delay of more than three or four minutes between the entry of an insect into the trap and its falling into the killing-bottle. As the minimum length of any period was 50 minutes this delay is of no significance.

Error from using a single trap as sample.

There is no doubt that more reliable results could be obtained by the use of several traps working simultaneously, and if the present investigation is to be continued this change would be recommended. On several occasions, however, a second trap has been in use at the same time as the standard trap. In September and October 1934 the two traps were working about 250 yards apart with almost identical conditions of illumination and outlook.

Fig. 1 shows the relation of the total catches to each other on each night

that the traps were working. The mean log. catch was 2.11 for trap A (the standard trap) and 1.91 for trap B (the additional trap) over the two months. The correlations for all insects was 0.86 for September and 0.92 for October.

In September only the correlation was 0.85 for TIPULIDAE; 0.97 for JASSIDAE, and 0.80 for NOCTUIDAE. Thus it will be seen that there is a very close relation between the two.

A statistical examination of the departures of each day's log. catch from the monthly mean in the two traps during the two months showed the mean square of the difference to be 0.0981. The mean square of the sampling error of a single trap is therefore 0.0491, and the actual sampling error is the square root of this or 0.222. By the method of differences between successive days the mean square of the sampling error is 0.0686. These figures will be used later in the analysis of variance.

Error from unit of measurement.

As will be shown below there is much evidence that changes in numbers in the catch are of equal significance when they are in the same geometric ratio. When the number of insects dealt with is large a single insect, which is the unit of measurement, is small in comparison with the total; but when dealing with a rare insect, or with times when insects are rare the unit of measurement may be such a high proportion of the total as to mask small changes; for example, when only four insects are in the trap the smallest possible reduction is one insect, or 25% of the total.

This error can only be avoided by dealing as far as possible with common insects, by increasing the number of traps, or by dealing with periods of the year when insects are most common.

Error due to distance of the meteorological station from the trap.

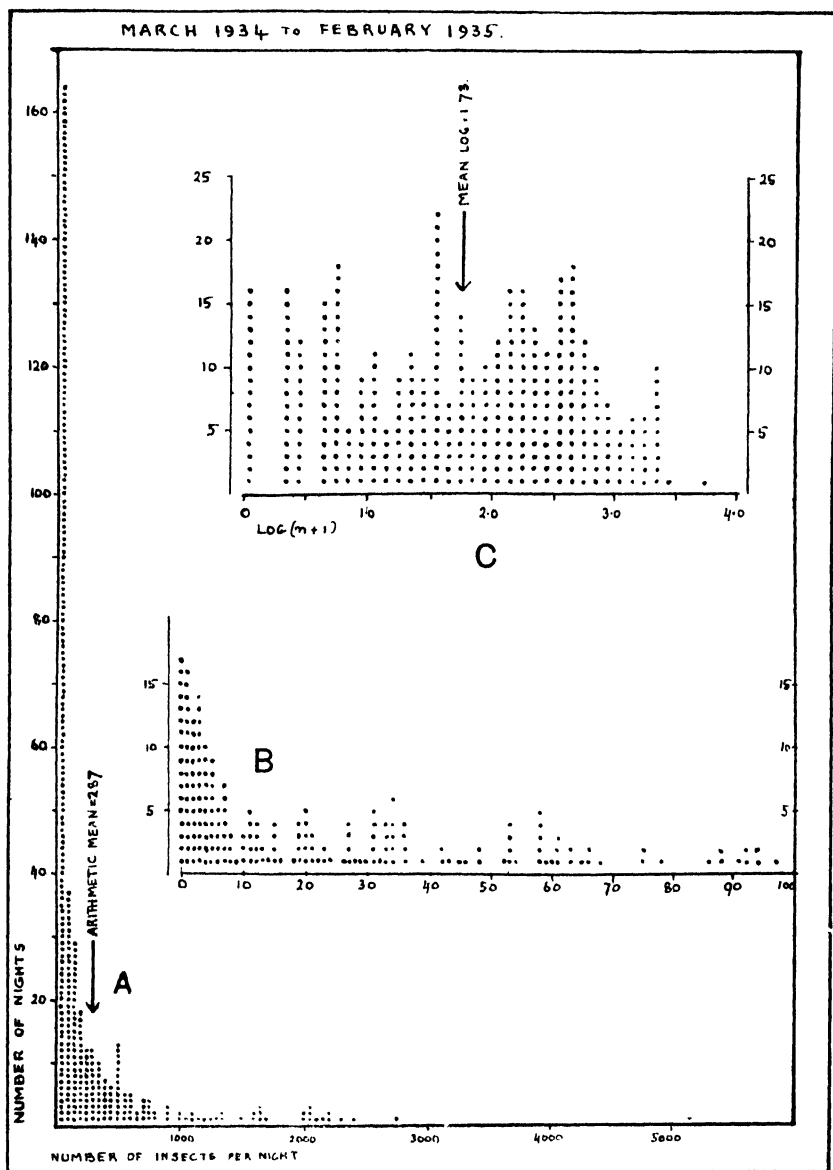
Owing to the fortunate fact that the meteorological station is only about 250 yards from the trap, the figures obtained for temperature and rainfall, etc., must be a very close indication of the conditions round the trap itself.

With wind (see p. 278) there is the possibility of a greater error, as the wind records are taken above the roof of the laboratories about 320 yards away to the east, at a height of about 70 feet from the ground, and so in a considerably more exposed position.

The use of geometric means in analysis.

When the catches in the trap night by night over a period of a year are examined it is found that they consist of a large number of small catches and a small number of large catches. Fig. 2, A shows the frequency distribution of the catches over a complete year from March 1934 to February 1935, arranged in groups of 0-49, 50-99, 100-149, etc. It will be seen that there were 164 nights with fewer than 50 insects, 37 nights with 50-99 insects, etc., and only one night with over 5000 insects. Fig. 2, B shows all the values below 100 arranged in units, from which it will be seen that the peak of the curve is at the zero value.

When the arithmetic mean catch per night (which in this year is 287 insects) is placed on the diagram it will be seen that there are a large number of nights below the mean with small negative departures and a small number of nights above the mean with large positive departures. There are, in fact, 265 nights below and only 95 nights above the arithmetic mean.



2

FIG. 2.—Frequency distribution of catches of all insects during 1934-35 :—
 A = all catches expressed as numbers in groups of 50.
 B = all catches up to 100 per night.
 C = all catches expressed as $\log(n+1)$.

In some of the other years the differences were even greater; for example in 1935-36 there were 181 nights with fewer than 50 insects, and 16 nights with over 5000 per night. Their distribution was 310 below the arithmetic mean (1102) and only 51 above.

It is of course impossible to use the normal formula relating to standard deviation, probable error, correlation and regression for figures showing a strongly asymmetrical distribution of this type.

It was, however, found that a better distribution could be obtained by considering the geometrical ratio between catches, rather than their arithmetic difference, and by using geometric means instead of arithmetic means. A fuller account of the application of the principle has been already published (Williams 1937).

The simplest method of obtaining such results is to deal with the logarithm of the catch number instead of the actual number itself. A difficulty arises, however, from the fact that not infrequently a zero catch is obtained. The log of zero is "minus infinity" and the geometric mean of any series containing zero is itself zero. To overcome this difficulty it has been found practicable to add unity to each of the catches before taking the log: *i.e.* using $\log(n+1)$ instead of $\log n$, where n represents the catch. These values can be summed or averaged for comparison, but should it be desired at any time to convert back to numbers (to find the geometric mean value) then unity must be subtracted from the anti-log.

If this process is used for the plotting of the distribution of the catches in each night of 1934-35 the result is as shown in fig. 2, C where the values of $\log(n+1)$ are divided into groups differing by 0.1. The first column on the left represents the 16 nights with no catch (as $\log(0+1) = 0$); the next column represents the 16 nights on which one insect only was caught each night (as $\log(1+1) = 0.30$), etc. The mean log value is 1.73 as indicated in the diagram by the vertical arrow.

It will be seen that distribution of the nights is much more normal and evenly placed about the mean. There are in fact 171 values below the mean and 190 above, and the extent of distribution on either side of the mean is almost identical. On a distribution of this type it is possible to use the normal formulae for correlation, etc.

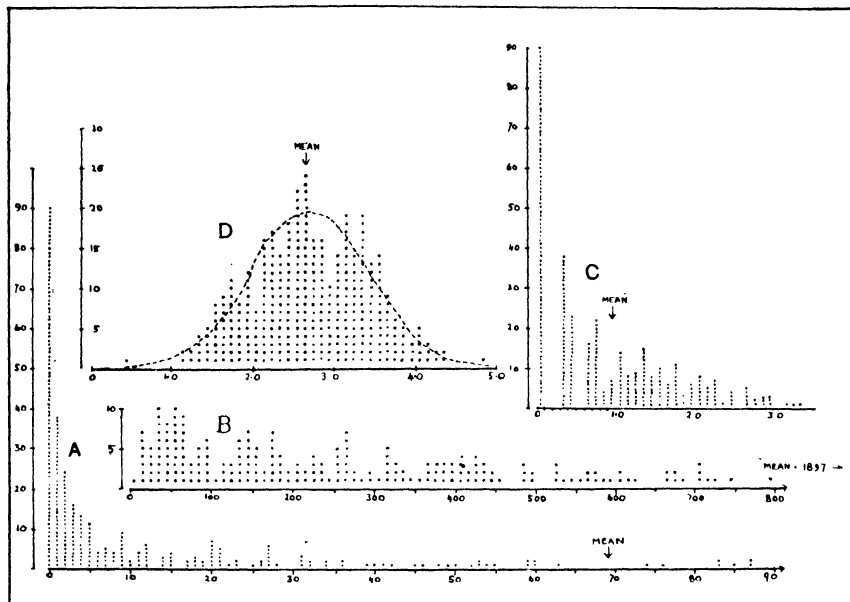
The shape of the curve of distribution of the catches is, however, not constant throughout the year, but changes from month to month. An examination of the results for each month separately has shown that the peak of the curve is at zero in March; at about 2 in April; about 5-9 in May; about 40-50 in June; about 80-90 in July; about 40-50 in August; 50-60 in September; about 11 in October; about 2-4 in November; and at zero again in December, January and February.

That is to say, that in the summer months there is a skew curve but in the winter the skewness has become so extreme that the portion below the peak at zero no longer exists. This is of course owing to the fact that it is not possible to catch a "fraction" of an insect in the trap. Theoretically the "zero" nights would include all nights on which conditions would lead one to expect between 0 and 1 insects (or only one insect in several traps).

To show this effect distinctly, fig. 3 has been prepared which gives the distribution in the three winter months (December, January and February) and the three summer months (June, July and August) of all four years together. Fig. 3, B shows the frequency distribution during the summer months for all catches below 800 per night arranged in groups of 10 units. The peak of the

curve is somewhere between 40 and 80 insects per night; the arithmetic mean of all catches is 1897. Fig. 3, A shows the distribution for the three winter months for all catches up to 90 in single units. The peak of this curve is at zero. Higher values of course continue in both these diagrams towards the right—in the case of the summer curve 3, B, up to a maximum of 73,000, which on the same scale would be approximately twenty-five feet away!

Fig. 3, D shows the frequency distribution of the logs ($n + 1$) of all the summer values, including all those off the previous diagram (3, B), and gives an almost perfectly normal distribution. The mean log value is 2.7 (exactly



3

FIG. 3.—Frequency distribution of catches of all insects :—

- A Three winter months in four years; expressed as numbers in units up to 90.
- B Three summer months in four years; expressed as numbers in groups of 10 up to 800.
- C Three winter months in four years expressed as log. ($n + 1$).
- D Three summer months in four years expressed as log. ($n + 1$) with superimposed normal curve of same area.

at the peak of the distribution) and the standard deviation is 0.716. From these data a normal curve of the same area has been calculated and superimposed as shown by the broken line. From this it will be seen how very closely the frequency distribution of $\log n + 1$ agrees with the curve of normal distribution during the summer months.

The winter values when transformed to logs do not give a distribution so obviously close to the normal, as is seen in fig. 3, C. The peak is at zero and the mean 0.9. It should be noted, however, that the high numbers at 0.3 and 0.4 are flanked by values with no representatives, and the large number of values at zero really represent (as has been mentioned above) all the fractional

values below one insect which cannot occur. Theoretically they would be spread out between 0 and $-\infty$.

It would appear, therefore, that the use of logs is an almost perfect transformation during the summer months when catches are high, but that it is not perfect (although at the same time much better than the actual numbers) during the winter months when dealing with low captures.

It follows from the above that if catches are at any time varying round a mean, then catches of twice the mean are as likely to occur as catches of half the mean; catches of three times the mean are as frequent as catches of one-third the mean; and so on.

As a result of the use of geometric means two valuable effects are obtained. First the swamping effect of occasional very large catches is greatly reduced, and secondly consistency in results is emphasised.

For example, a comparison was made (Williams 1937) between the total captures in the same trap on all the odd nights and all the even nights in the first three years. There can be no possible reason to expect a difference between these two sets of values yet, owing to the accidental distribution of a few very large captures, the arithmetic mean catch of the odd nights was 45% higher than that of the even nights. When the geometric mean was used the difference between the odd and even nights was reduced to 4.5%. Thus a difference of 45% in actual numbers after over 1000 nights (500 comparisons) was of no significance.

A second example may be quoted in the number of moths of the family NOCTUIDAE captured each night in the week of full moon and in the week of no moon, in October 1933 (see Williams 1936a) as shown in Table 3.

TABLE 3.

Catches of NOCTUIDAE in the week of full moon and the week of no moon in October 1933, on number and logarithm basis.

Day of week	Number		Log ($n + 1$)	
	No moon	Full moon	No moon	Full moon
1	2	0	0.48	0
2	4	0	0.70	0
3	0	1	0	0.30
4	0	62	0	1.80
5	10	0	1.04	0
6	3	0	0.60	0
7	3	0	0.60	0
Mean log	—	—	0.49	0.30
Mean no. per night .	3.1	9.0	2.1	1.0

It will be seen that on the arithmetic basis the mean catch in the full-moon week is 9 and in the no-moon week 3.1. The higher value for the full-moon week is, however, almost entirely due to the abnormal catch of 62 insects on one night (which was heavily clouded). When the log ($n + 1$) is used the geometric mean catch is found to be 2.1 insects in the no-moon week and 1 in the full-moon week; thus giving a higher value in the no-moon week. This latter result is consistent with a large number of other comparisons between full- and no-moon weeks.

On the other hand, when consistent differences are present, the use of logs shows them most distinctly. For example, the ratio of the arithmetic mean catches in the first and last periods of the night of all insects in 1933 was 49.0 to 26.3 (see Williams 1935 : 531), while the ratio of the geometric means was 7.3 to 3.6. The former is 182 : 100 and the latter 203 : 100, thus showing a greater difference with the logs, owing to consistent differences in the original figures.

Several other examples of the use of the technique are discussed in the paper already quoted (Williams 1937), and it is generally adopted in the remainder of this paper.

What is the catch ?

The catch in the light trap in any one night is a sample—as unbiased as possible—of the night-flying positively phototropic insects which are active in the neighbourhood of the trap. The area from which these are drawn is not known, nor is it likely that this area is the same for different species, or for the two sexes of the same species, or even for the same insect on different nights.

If the night is cold the majority of insects will be dormant and lie hidden away in sheltered places as they have during the day : the catch will then be small. If the night is warm there will be greater activity and a correspondingly greater catch. The catch therefore is directly affected by the state of activity of the insects.

If, on the other hand, the activity of the insects is the same on two nights, but the total number of insects in the district is different, then again the catch will alter.

The catch is therefore proportional to both the activity and the population of the phototropic insects ; otherwise :—

$$\text{Catch} \propto \text{Activity} \times \text{Population.}$$

The activity is, however, affected by a number of different factors such as wind, rain, temperature, humidity, etc., each of which can apparently act independently of the others. Thus :—

$$\text{Total activity} = \text{activity due to temperature} \times \text{activity due to wind} \times \text{activity due to humidity etc.}$$

Therefore :—

$$\text{Catch} \propto [\text{act. T.} \times \text{act. W.} \times \text{act. RH. etc.}] \times \text{Pop.}$$

Since, however, proportional changes in the catch, as expressed by logs, are being dealt with almost entirely the result is :—

$$\log. \text{ catch} = [\log. \text{ act. T.} + \log. \text{ act. W.} + \dots] + \log. \text{ P.}$$

The log. catch is therefore made up of portions due to the effect on activity of the different environmental factors plus a portion determined by the total population available for sampling.

GENERAL SURVEY OF CLIMATIC FACTORS.

It is impossible to study insects in the field without realising to what a large extent their activity and abundance are determined by the effect of the various climatic factors of their environment. It is, however, a big step from the realisation that some of the factors have a large and some a smaller effect, to a proper analysis which will show on some scale of measurement exactly how

much effect is produced in the fauna by a particular extent of change in one particular factor of the environment.

One expects that if any change in a single factor, such as temperature, produces a change in the catch of the trap, then the size of the change in the catch (the dependent variable) will be in some way proportional to the change of the weather factor (the independent variable).

Thus in any locality where there is a big variation in temperature, the effect on the catch will be large; if, however, temperature changes in the environment are small, the effect will be small. If, however, it is possible to express the total effect in terms of the effect produced by *unit changes* in the independent factor, one will have a figure that can be used not only to compare effects from day to day in one locality but also to compare the effect of that factor in different localities where its variability is different and it is more or less influenced by other competing factors.

The object, therefore, is to attempt to express the effect of the weather factors of the environment on the catch (and later, on the population) as an integration of effect of a series of influences, the individual effect of each of which is a multiplication of the effect per unit change by the extent of the change.

The weather conditions which may be concerned can be conveniently grouped under six headings:—

- | | |
|------------------------|----------------------------|
| (1) Temperature. | (4) Wind. |
| (2) Radiation (Light). | (5) Atmospheric pressure. |
| (3) Moisture. | (6) Thunder and lightning. |

(1) *Temperature.*

Temperature is probably the most important single factor in the climate and weather of a locality such as Rothamsted, in the cool temperate zone (latitude 51° 50' N.). Temperatures are taken as part of the routine of the meteorological section, and the principal ones that have been considered are the maximum air temperature of the day previous to the catch and the minimum during the night of the catch, which normally occurs about dawn during the last of the eight periods into which the catch was automatically divided. These temperatures are taken in a screen at a height of about 4 feet from the ground and so almost exactly at the level of the trap.

The mean difference between these two, which is the "daily range," varies from 10° to 17·7° F. in different months, but this has not been found to have any very close connection with the catches in the trap (see pp. 246 and 268).

In addition to the above a minimum thermometer is exposed just above the surface of the ground, and the reading, known as the "grass minimum," gives a close measure of the lowest temperature to which the air in contact with the surface of the ground has fallen. The ground cools by radiation more rapidly on a clear night than on a cloudy night and so the differences between the air minimum and the grass minimum are related to the amount of cloud. Large differences are usually found on clear nights, while if the difference is 2° F. or less the night is certainly heavily clouded. Occasionally with complete cloud the grass minimum is a little above the air minimum.

It has been found undesirable as a general rule to consider the effect of temperature at any fixed hour, as the length of the night is continually varying and the 9 p.m. reading is, for example, in some months before the trap starts and in others after it has been working for several hours. However, in one

or two cases the effect of 9 p.m. temperature has been studied for June and July when the length of the night is varying less rapidly.

(2) *Radiation.*

The source of heat which alters the air temperature is, of course, radiation from the sun. The light from the sun does not directly affect the catches as trapping only starts after sunset and ends before sunrise. It will be shown later that there is a very slight tendency for good catches to follow days with more sunshine, but this is probably an indirect effect through temperature.

Moonlight has, however, a definite reduction effect on the catch, which has already been discussed in a previous paper (Williams 1936a). The catch is affected by both intensity and duration of moonlight. The duration of moonlight during the trap hours depends on the hours at which the moon rises and sets in relation to the hours of darkness. The intensity of moonlight depends upon the phase of the moon and on its angle above the horizon.

The time of rising and setting of the moon is on an average 49 minutes later each night, but the interval varies from 12 minutes to 1 hour and 33 minutes, and is of course not the same for rising and setting on the same night.

The amount of moonlight reaching the ground is much affected by the prevalence of cloud.

(3) *Moisture.*

In normal meteorological records rain is recorded as between 9 a.m. and 9 a.m. Thus any rain that falls up to 9 a.m. on one day is credited to the previous day. These figures may be misleading if attempts are made to use them in a calculation of the present type.

Rain is, however, measured at Rothamsted by recording instruments which give the time and amount of precipitation, and from these charts figures have been obtained for the rainfall from 6 a.m. to 6 p.m. and from 6 p.m. to 6 a.m. separately. These are known as the "day" and "night" rain.

The amount of water in the atmosphere can be expressed in three different ways:—

- (a) as the weight of water vapour in a given volume of air; usually expressed as grammes per litre;
- (b) as the vapour pressure of the water vapour, usually expressed in millimetres or inches of mercury;
- (c) as "Dew point," which is the temperature at which the particular amount of water in the air is sufficient to produce saturation.

In general, however, atmospheric moisture in biological work is not expressed in absolute units but in connection with temperature as either "Relative Humidity" or "Saturation Deficiency."

The former is a measure of the amount of water in the air in proportion to the total amount that would be required to produce saturation. The latter is the additional amount required to produce saturation.

Thus if VPT is the vapour pressure that would be required for saturation at the given temperature, and if VPD is the vapour pressure of saturation of the given air at the dew point, then

$$\text{Relative Humidity} = 100 \times \frac{\text{VPD}}{\text{VPT}}$$

and

$$\text{Saturation Deficiency} = \text{VPT} - \text{VPD}.$$

The relation between Relative Humidity and Saturation Deficiency is linear

for a single temperature, but does not remain the same for a change of temperature.

Still another measure including both moisture, temperature and wind is "evaporation," but owing to the lack of any good standardisation of this in the meteorological observations it has not been used in the present work.

There have been for many years differences of opinion on the relative value for biological work of the use of Relative Humidity and Saturation Deficiency and this point will be discussed in the statistical analysis which follows.

Wet and dry bulb readings, from which both R.H. and S.D. values can be calculated, were taken at Rothamsted three times a day, at 9 a.m., 3 p.m. and 9 p.m. in the first year and a half of the trap work; but unfortunately in July 1934 the 3 p.m. and 9 p.m. readings were discontinued. Records after this date have to be calculated from recording hygrometers which were not very reliable. The relative humidity at midnight and after is nearly always over 90% even in the summer months.

Other factors associated with atmospheric humidity are cloud and fog.

Cloud, in addition to indicating high humidity, has two effects already referred to: (1) it prevents radiation of heat from the ground at night and so helps to keep up the night temperatures; (2) it keeps off radiation from the sun during the day and the moon during the night. Cloudy nights in general differ from clear ones in having lower maximum and higher minimum and grass minimum temperatures, but have about the same mean temperature. They have a much smaller daily range and a smaller difference between the screen minimum and the grass minimum.

Fog is only a special case of cloud at ground level, but in addition to indicating high moisture it has a new biological effect of interfering with visibility. It occurs usually on cold nights with clear sky in the winter, but occasionally warm fogs occur early in the autumn. (See p. 271.)

(4) *Wind.*

Wind is recorded at Rothamsted by a Dines Anemograph situated on the roof of the laboratory about 320 yards from the trap, the vane being at a height of about 70 feet.

The analysis of wind is made difficult by its rapid variation in strength, sometimes one part of a night being stormy and another part calm. This is particularly the case in winter, when nights frequently start stormy and become calm well after midnight. Fuller details will be found on p. 278.

(5) *Barometric pressure.*

Barometric pressure is recorded regularly, and for the purpose of most of the analyses below the days are divided into 9 categories, showing the interrelation of two sets of three factors: (A) barometer high, medium or low. (B) barometer rising, steady or falling. Full details will be found on p. 284.

(6) *Electrical phenomena.*

Thunder or lightning, or both, were recorded on forty occasions during the six summer months in the four years.

(7) *Interrelation of climatic factors.*

With so many possible factors the analysis of their effects is by no means likely to be easy, but the problem is unfortunately complicated even further by the close relation that many of them bear to one another.

In Table 3 an attempt has been made to summarise briefly some of these interrelations. A + indicates that the two factors rise and fall together; a — indicates that as one increases the other decreases.

Thus one may see, for example, that maximum temperature is positively associated with minimum temperatures, with the difference between minimum and grass minimum, with sunshine, and with the level of the barometer; but it is negatively associated with relative humidity, day rain, cloud and perhaps wind.

TABLE 4.
Interrelation of various weather factors.

	Max. temp.	Min. temp.	Min. g.m.	Sun	Moon	R.H.	Day rain	Night rain	Cloud	Wind
Temperature, min.	+	?								
Temperature, min.-G.M.	+	?								
Sunshine	+	?	+	+						
Moon (light reaching ground)	?	?	+	+						
Relative humidity	—	?	—	—						
Rain (previous day)	—	—	—	—		+				
Rain (during night)	—	+	—	+		+	?	+		
Cloud	—	+	—	?	—	+	+	+		
Wind	?	+	?	?	—	?	?	?	+	
Barometer (level)	+	+	?	+	?	—	—	—	—	—

Minimum temperature, on the other hand, is positively correlated with night rain, cloud, wind and barometer.

Although the phases of the moon are regular and independent of weather conditions, yet the amount of moonlight reaching the earth (and hence any effect on the trap) is negatively correlated with relative humidity, night rain, cloud and wind and positively correlated with sunshine and the differences between minimum and grass minimum temperature.

Windy nights tend to have higher minimum temperature than calm nights, and this is particularly marked in the winter.

Thus it is almost impossible by simple means to get at the exact effect on the catch of each factor, independent of all others. The only method that seems capable of giving an answer is the statistical method of multiple partial regressions, which will be discussed later.

The simple relation of catch to any one factor is, however, often of considerable interest to the biologist, and the plan adopted will be to deal first with the direct relationships of single factors and then with the analysis of multiple effects.

COMPARISON OF NIGHTS OF HIGH AND LOW CATCH.

As a preliminary to a more detailed survey of single factors, it is interesting to make a comparison of the weather conditions prevailing on nights with unusually high or unusually low catch. To provide data for this the three nights with the highest catch and the three with the lowest in each of the months from May to October in each of the four years have been tabulated. That is to say, 72 good and 72 bad nights spread over the summers of four

years, each group containing approximately 10% of the total nights in that period.

TABLE 5.

Examples of the weather conditions on days with unusually high and unusually low catches in the summer of 1935.

Temperature. m.-g.m. = difference between screen minimum and grass minimum.
Night cloud. H = heavy cloud (over 90%), I = intermediate cloud (10-90%), L = light cloud (less than 10%).
Moon. a = after, b = before, N = new moon, F = full moon.
Wind. number in 6 groups as explained in text.
Barometer. R = rising, S = steady, F = falling; H = high, M = medium, L = low.

1935	High catch						Low catch					
	June		July		Aug.		June		July		Aug.	
	23	24	2	10	7	8	8	11	8	18	13	27
Catch, log. depart. from mthly. mean	+1.12	+1.20	+0.67	+0.80	+0.82	+0.89	-1.06	-1.01	-0.88	-1.32	-1.39	-1.07
Temperature:												
Max. . .	78.9	81.5	76.9	79.3	82.0	80.0	61.2	60.8	69.0	62.2	65.2	61.4
Min. . .	60.2	60.5	56.2	61.5	57.4	58.5	41.5	47.5	49.6	45.4	46.1	40.2
Grass min. .	52.3	57.6	53.3	55.8	51.3	54.3	34.1	43.4	40.8	40.7	38.4	33.3
Range . .	18.7	21.0	11.7	17.8	24.6	21.5	19.7	13.3	19.4	16.8	19.1	21.2
Min.-g.m. .	7.9	2.9	2.9	5.7	6.1	4.2	7.4	4.1	8.8	4.7	7.7	6.9
Night cloud .	L	I	I	I	II	II	I	L	L	L	L	I
Moon, pos. in cycle .	7aF	6bN	2aN	5bF	7bF	6bF	8aN	5bF	7bF	3aF	1bF	1bN
Wind, group .	1	1	3	1	1	1	2	5	1	1	1	1
Sun, hours .	14.3	15.2	8.6	12.8	9.1	5.3	13.1	5.6	13.9	2.5	11.5	5.3
Rain, day .	—	—	—	—	—	—	—	0.06	—	0.62	—	—
Rain, night .	—	—	—	—	—	0.05	—	0.01	—	—	—	—
Barometer .	MF	MF	HR	MR	MF	MF	MR	LR	HF	LS	MR	MF
Rel. humidity:												
Min. prev. day .	50	53	64	47	44	47	52	60	51	81	43	72
Sunset . .	80	80	85	66	64	59	68	95	76	94	62	88
9 p.m. . .	81	85	88	72	78	72	75	93	84	83	74	92
Midnight .	92	95	87	85	84	78	92	96	94	92	86	94
Sunrise . .	95	96	93	92	94	91	98	96	95	95	92	94
Max. of night .	95	96	93	92	94	91	98	96	95	95	92	94

Table 5 shows a sample of the data for 1935, including the departure of the log. catch from a 29-day running mean, various temperatures, and measures of cloud, moon, wind, sunshine, rainfall, barometer and relative humidity at various hours.

Tables 6, 7, and 8 show a summary of the average values of these factors on the best and worst nights for each year, in all four years, and the differences between the values. Figs. 4 and 5 show the frequency of occurrence of the values of certain of these factors in the two opposing types.

In the first place it will be seen from Table 6 that the average departures of the log. catch on all good nights is +0.98 above normal, and on the bad nights -1.02 below normal. Converted into numbers, this means that the 72 good nights have catches averaging almost exactly ten times the normal, and the 72 bad nights have catches almost exactly one-tenth of the normal. It may be noted in passing that this great similarity of log. departures for the two extreme groups, each containing 10% of the total observations, is a further strong argument for the validity of the use of logarithms in analysis.

In the maximum temperature the good nights average about 9° F. above the bad nights with somewhat similar variability in each group (fig. 4, A).

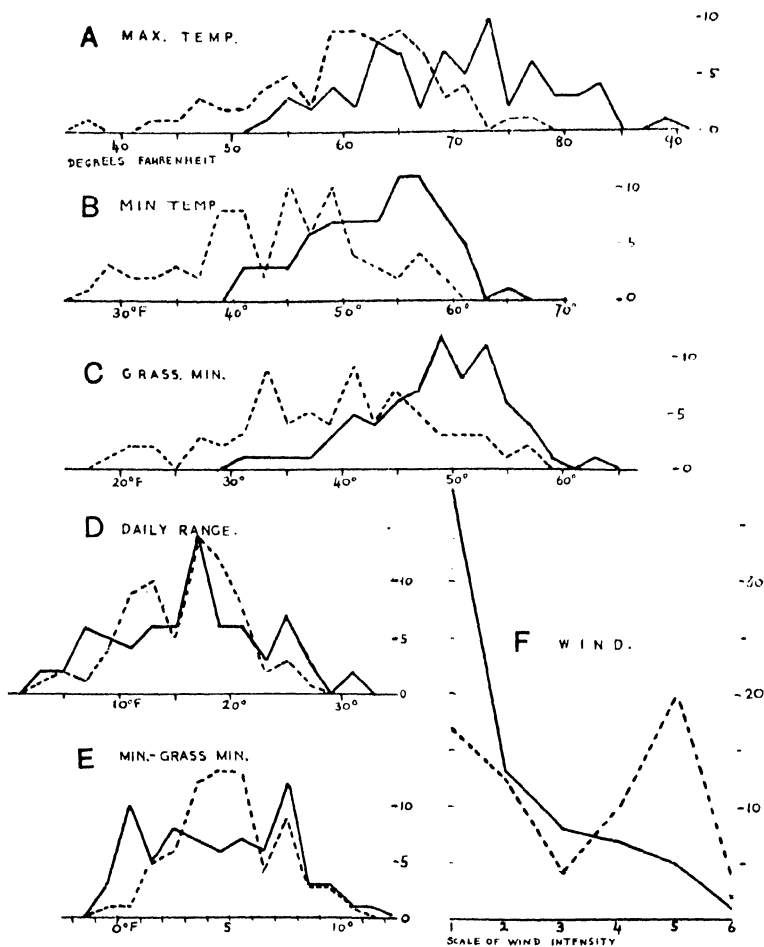
In the screen minimum temperature the good nights average 9.5° F. above the bad nights, but it will be seen from fig. 4, B that, in addition to the difference

TABLE 6.
Difference between certain weather conditions on nights of high and low catch in six summer months in all four years.

Year	Catch	Temperature					Night cloud			Sun Hours	Rain					
		Max.	Min.	Grass min.	Daily range	m.-g.m.	No. of days				Day		Night			
							Clear	Int.	Cloudy		Tot.	No. of days	In. per day	Tot.	No. of days	In. per day
Best nights :																
1933	+0.99	71.5	54.3	49.5	17.5	4.8	3	7	6	7.2	2	0.15	0.07	4	—	
1934	+0.89	70.7	53.5	49.2	17.2	4.3	3	9	6	5.6	1	0.01	0.12	3	—	
1935	+1.11	70.2	52.0	47.3	17.6	4.7	4	7	6	6.7	4	0.76	0.71	5	—	
1936	+0.91	65.2	51.9	47.7	13.5	4.2	1	7	10	4.1	4	0.69	0.56	6	—	
4 years	+0.98	69.4	52.9	48.4	16.5	4.5	11	30	28	5.9	11	1.61	1.46	18	0.08	
Worst nights :																
1933	-1.09	63.0	47.2	43.1	15.8	4.1	5	10	3	6.8	9	0.60	0.17	4	—	
1934	-0.89	60.3	42.8	37.6	17.5	5.2	13	0	3	4.5	7	1.10	0.79	5	—	
1935	-1.04	58.5	41.6	35.7	16.9	5.9	11	6	0	6.2	7	0.99	0.60	4	—	
1936	-1.05	59.0	45.4	41.1	13.6	4.3	8	6	4	3.8	11	1.45	0.49	6	—	
4 years	-1.02	60.2	43.4	39.4	16.0	4.9	37	22	10	5.3	34	4.14	2.05	19	0.11	
Difference :																
1933	+2.08	+8.5	+10.5	+6.4	+1.7	+0.7	—	—	—	+0.4	—	—	—	—	—	
1934	+1.78	+10.4	+10.7	+11.6	-0.3	-0.9	—	—	—	+1.1	—	—	—	—	—	
1935	+2.15	+11.7	+10.4	+11.6	+0.7	-1.2	—	—	—	+0.5	—	—	—	—	—	
1936	+1.96	+6.2	+6.5	+6.6	-0.1	-0.1	—	—	—	+0.3	—	—	—	—	—	
4 years	+1.99	+9.2	+9.5	+9.0	+0.5	-0.4	+26	+8	+18	+0.6	-23	—	—	-1	—	

in the average value, the minimum temperatures on the good nights are distinctly less variable than those on the bad nights.

The conditions of "grass minimum" temperature closely resemble those of minimum temperatures, with a mean difference of 9° F., and with less variability on the good nights (fig. 4, C).



4

FIG. 4.—Frequency distribution of occurrence of various weather conditions on nights of unusually high catch (solid line) and on nights of unusually low catch (dotted line) during six summer months (May to October) of four years.

The mean daily range (fig. 4, D) on good nights is almost identical with that on bad nights, but their distribution is different. The ranges on the bad nights are more closely grouped about the mean and less variable than the ranges on good nights.

In the difference between the screen minimum and the grass minimum (fig. 4, E), which (as has been explained) is a measure of the radiation of heat from the earth during the night, there is again practically no difference between

the average value on the good and bad nights, but the distribution is very different. The values on the poor nights are mostly grouped round the mean, whereas on the good nights there is evidence of two peak values, one with practically no difference between the two minima and the other at a difference of about 8° F. The former are nights of heavy cloud; the latter are apparently relatively clear nights in which the high catch has been produced by exceptionally high minimum temperatures probably following very hot days.

The distribution of night-cloud is quite different in the two types of night (Table 6). The 72 good nights include 28 with over 90% cloud and only 11 with less than 10% cloud. The poor nights, on the contrary, include only 10 with more than 90% cloud and 37 with less than 10%.

The wind for the purpose of comparison has been grouped into six artificial categories with maximum velocities during the night as follows: (1) nil, (2) less than 2 m.p.h., (3) 2-5, (4) 5-10, (5) 10-20, and (6) over 20 m.p.h. (see p. 278).

Table 7 and fig. 4, F show the frequency distribution of the different wind forces on good, on bad and on all nights. It will be seen that, as might be expected, dead calm conditions are much more frequent on the good nights and winds of over 10 m.p.h. more frequent on the poor nights. It is curious to note, however, that with a wind as slight as group 2 (under 2 m.p.h.) there is little or no difference between the good, the bad and normal nights.

The effect of moon has already been discussed in a previous paper (Williams 1936a). Fig. 5, G shows graphically how the good and poor nights are distributed according to the position in the lunar cycle. It will be seen that the good nights are more frequent in the new-moon half of the cycle and the bad nights in the full-moon half.

TABLE 7.

Frequency of occurrence of different wind intensities on nights of high and low catch.

	Wind group					
	1	2	3	4	5	6
Best nights	38	13	8	7	5	1
Worst nights	17	12	4	10	25	4
All nights	26	11	8	11	11	2

Table 6 shows that the days preceding good nights have an average of about 0.6 hours more sunshine than the days preceding poor nights. The difference is small and of doubtful significance, but it should be noted that each of the four years taken separately shows a positive difference in favour of good nights. Fig. 5, H indicates that there is little or no difference in distribution and with such a large range the small difference is inconspicuous.

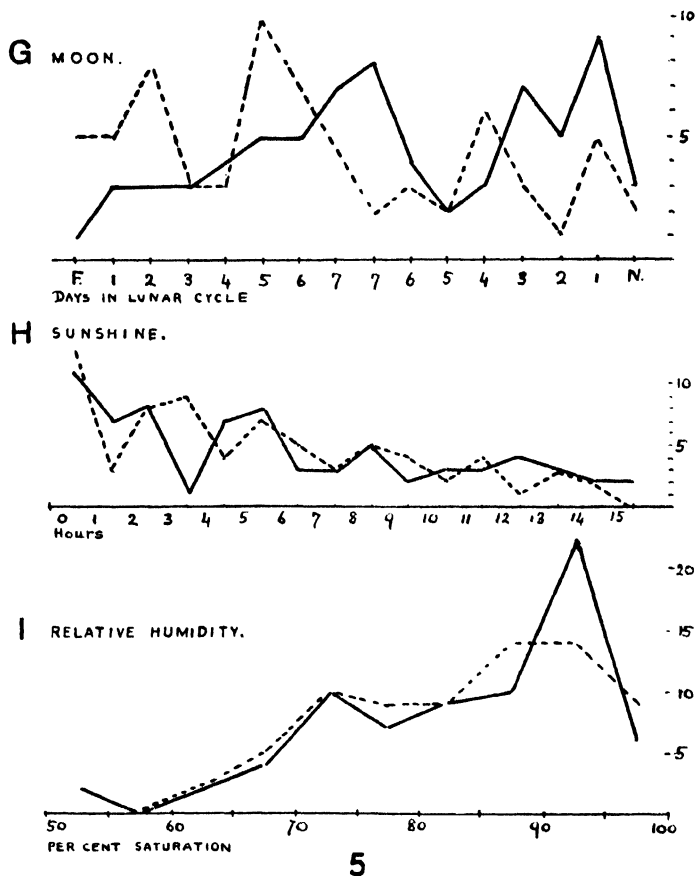
The sunshine problem is connected with that of rainfall which shows an interesting difference.

Separate figures are fortunately available for rainfall during the twelve day-hours 6 a.m. to 6 p.m. and during the 12 night-hours 6 p.m. to 6 a.m. Table 6 shows that night rain occurred on 18 of the best nights (with an average fall of 0.08 inches per night) and on 19 of the worst nights (with an average of 0.11 inches per night). There is practically no difference in these.

In the 12 hours daytime preceding the catch, however, rain fell on only 11 of the 72 best nights, but on 34 of the worst nights; that is, practically

half of the worst nights followed days with rain. Therefore it appears that rainfall during the daytime is associated with a low catch on the following night, but rain which falls during the night seems to have little or no effect.

The distribution of the good and bad nights in different conditions of barometric pressure is shown in Table 8. The barometer is divided into 9



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FIG. 5.—Frequency distribution of occurrence of moon phase, sunshine and relative humidity on nights of unusually high catch (solid line) and unusually low catch (dotted line) during the six summer months (May to October) of four years.

TABLE 8.

Frequency of occurrence of different barometrical conditions on nights of high and low catch.

	Best				Worst				Difference (best - worst)			
	R	S	F		R	S	F		R	S	F	
High .	8	11	1	20	2	2	3	7	+ 6	+ 7	- 2	+ 13
Medium .	8	16	22	46	11	8	15	34	- 3	+ 8	+ 7	+ 12
Low .	3	2	1	6	18	6	7	31	- 15	- 4	- 6	- 25
	19	29	24		31	16	25		- 12	+ 13	- 1	

categories according to whether it is on the one hand high, medium or low, and on the other hand rising, steady or falling.

An examination shows that high catches are more frequently associated with a high barometer if rising or steady, and with a medium barometer if steady or falling; low catches are associated with low barometer either rising, falling or steady, with a medium barometer if rising, and unexpectedly, with a high barometer if falling. This latter point will be discussed more fully later (see p. 284).

If only the height of barometer is considered, good catches are more frequent with high or medium barometer and poor catches with low barometer. If only the direction of movement is considered, low catches are more frequent with a rising barometer, high catches with a steady barometer, and both are equally frequent on a falling barometer.

The problem of atmospheric humidity is particularly complicated as there are so many ways by which the results can be expressed. Table 9 shows a summary of comparisons of Relative Humidity at different times of the day and night. These include the minimum humidity of the previous day, the humidity at sunset, 9 p.m., midnight, sunrise and the maximum humidity during the night. In no case is there any significant difference between the good nights and the bad nights.

It might be inferred from this that there is no selection of damp or dry nights for high insect activity. This interpretation is not, however, correct. It has already been shown that the best nights are about 9° F. warmer than the poorest nights. Warm nights, however, have on an average a lower relative humidity than cool nights. Since there is *no* difference in the average relative humidity between the good (warm) nights and the poor (cold) nights it follows that good nights must be associated with the damper of the warm nights and poor nights with the dryer of the cool nights. Therefore the absence of a difference in relative humidity indicates a selection of nights of higher relative humidity for high activity (see p. 268).

TABLE 9.

Conditions of relative humidity at different times of night on nights of high and low catch.

Year	Min. prev. day	Sunset	9 p.m.	Midnight	Sunrise	Max.
Best nights						
1933 . .	—	—	79	—	—	—
1934 . .	58	69	78	83	87	90
1935 . .	62	79	86	89	94	94
1936 . .	75	87	91	95	95	97
4 years .	65	78	84	89	92	94
Worst nights						
1933 . .	—	—	80	—	—	—
1934 . .	62	74	78	90	93	95
1935 . .	60	78	85	90	94	95
1936 . .	71	86	90	95	95	97
4 years .	64	77	83	92	94	96
Difference						
1933 . .	—	—	—1	—	—	—
1934 . .	—4	—5	0	—7	—6	—5
1935 . .	+2	+1	+1	—1	0	—1
1936 . .	+4	+1	+1	0	0	0
4 years .	+1	+1	+1	—3	—2	—2

THE GENERAL METHODS OF ANALYSIS OF THE INFLUENCE OF A SINGLE FACTOR,
AND THE RELATION OF TOTAL CATCH TO MINIMUM TEMPERATURE.

In order to explain the various problems in connection with the mathematical analysis of the influence of a single factor on the catch in the light trap, it is necessary to give a number of examples of typical methods and results. It has been thought desirable to use for this discussion examples relating to the action of minimum night temperature on total catch, so that this section will include both the discussion of methods and of the effect of this factor of the environment.

Before discussing other problems an attempt will be made to explain the exact meaning of the terms "regression," "correlation" and "variance" which are the bases of most of the analyses to follow, so that the reasoning can be followed by entomologists and others who are not specially familiar with statistical methods and nomenclature.

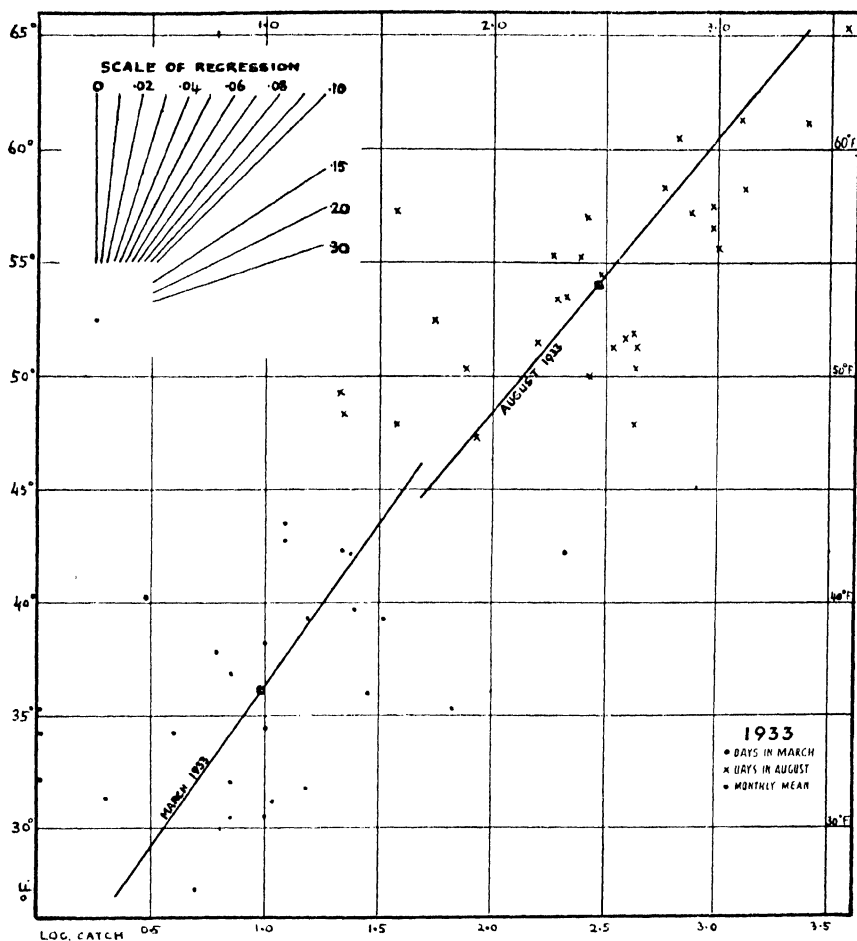
Table 10 shows—as an example—the catch, the log. catch, and the minimum temperature on each night of the months of March and August 1933. It will be seen that in March the mean log. catch was 0.97 and the mean minimum

TABLE 10.

Catch, log. catch and minimum temperature on the nights in March and August 1933.

Date	March 1933			August 1933		
	Catch	Log. catch	Min. temp.	Catch	Log. catch	Min. temp.
1	31	1.51	39.3	424	2.63	50.3
2	208	2.32	42.3	3596	3.56	65.3
3	—	—	—	1249	3.10	61.4
4	—	—	—	569	2.76	58.4
5	—	—	—	262	2.42	57.0
6	66	1.83	35.4	2500	3.40	61.2
7	9	1.00	34.5	661	2.82	60.5
8	22	1.36	42.1	371	2.57	51.6
9	27	1.45	36.0	992	3.00	55.6
10	6	0.85	36.8	235	2.37	55.2
11	3	0.60	34.3	192	2.29	53.3
12	6	0.85	32.1	74	1.88	50.4
13	2	0.48	40.2	209	2.32	53.5
14	9	1.00	38.3	939	2.97	57.5
15	11	1.08	43.5	179	2.26	55.3
16	11	1.08	42.8	83	1.92	47.3
17	5	0.78	37.9	35	1.56	57.3
18	14	1.18	39.3	54	1.74	52.5
19	—	—	—	159	2.20	51.5
20	4	0.70	27.3	20	1.32	49.5
21	10	1.04	31.2	21	1.34	48.4
22	0	0	34.3	267	2.43	50.0
23	0	0	32.1	35	1.56	47.9
24	1	0.30	31.3	432	2.64	51.4
25	—	—	—	420	2.62	51.8
26	9	1.00	30.5	335	2.53	51.3
27	14	1.18	31.8	740	2.87	57.3
28	6	0.85	30.5	949	2.98	56.5
29	23	1.38	39.7	1295	3.11	53.3
30	0	0	35.3	426	2.63	47.8
31	21	1.34	42.3	301	2.48	54.5
Mean	20	0.97	36.2	581	2.46	54.2

temperature was 36.2° F.; while in August the values were 2.46 and 54.2° F. respectively. It will also be seen that in general the larger catches are on the warmer nights and the smaller catches on the cooler nights. The night of the largest catch in August—3596 insects on the 2nd of that month—had a minimum temperature of 65.3° F., which was the highest experienced in all the four years of work.



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FIG. 6.—Scatter diagram and regression lines showing relation of log. catch of all insects and minimum temperature of the nights in March and August 1933.

The results can be expressed graphically in the form of a “scatter diagram” for each month. In this (fig. 6) each night is represented by a dot in the position corresponding to its correct minimum temperature and log. catch. There are for March, 26 dots (five days were missing) in the lower portion of the figure, with a larger dot representing the mean catch and mean minimum temperature for the month. It will be seen that the dots are spread over an area elongated

in a direction which indicates that, as a general rule, the higher the minimum temperature the larger the catch.

Through such a diagram it is possible to draw a line along the axis of this area which is a "best fit" to the points. If the points are close to a straight line—that is to say, if the influence of temperature is very definite and uncomplicated by other effects—the line may be fitted easily by eye. But if (as is more usual) the dots are scattered, the line can be best fitted in by calculation, and is known as the line of regression. The method of calculation will be found in any elementary book of statistics.

The regression line, as found by calculation, has been fitted to each of the two months shown in fig. 6. It indicates the average rate of change of catch (the dependent variable) per unit change of temperature (the independent variable). The calculated regression is the value which most closely explains the available data and leaves the least variation to be explained (or perhaps not explained!) by other sources of influence not considered at the moment.

For example, in fig. 6 the calculated regression of log. insect catch on minimum temperature for March is $+0.052$. In other words, the data given for March are most completely explained on the assumption that a rise of one degree Fahrenheit in the minimum temperature is associated with an increase of 0.052 in the log. catch; and, of course, each degree of fall in temperature is associated with a corresponding fall in the catch.

The regression for August is $+0.084$, showing that, from the data available, a degree rise in August 1933 is apparently associated with a greater increase in catch than a degree rise in March of the same year. Whether these figures are significantly different will be discussed later.

Since an increase of 0.301 on the log. represents a doubling of the catch at any level, the above figures can be restated by saying that in March 1933 the catch would be doubled with an increase of 6°F. in the minimum temperature, and in August 1933 with an increase of 3.6°F.

The angle of the line of regression, when expressed graphically, alters as the value alters, and an angular scale has been added to fig. 6 to illustrate this. The values on this scale, of course, depend on the relative scales of the horizontal and vertical co-ordinates.

A second value that can be calculated from the figures is the "correlation" between the two sets of values. This is to a certain extent a measure of the closeness of fit of the individual points to the regression line. A correlation of $+1.0$ shows that the relation between the two factors is complete, *i.e.* all the points are exactly on the regression line, and that as the value of one factor rises the other rises in exact proportion. A correlation of $+0.5$ indicates that the points are somewhat scattered. A correlation of 0 indicates that there is no evidence of relation between the two factors. Negative correlations indicate that as one factor rises the other falls.

For example, the points for August 1933 in fig. 6 give a correlation of $+0.68$; those for March, which are more scattered, give a lower value of $+0.43$.

The significance of the correlation depends on the number of pairs of values from which it is calculated. The fewer the values the higher must be the correlation for the same significance. In the present analyses most of the figures have been grouped in months with about 30 or 31 days. For this number of observations a correlation must be above ± 0.35 to have a probability value of 0.05 (*i.e.* only once in twenty repetitions might the results be

obtained by chance), or above ± 0.41 to have a probability of 0.02 (*i.e.* only once in 50 repetitions might it be obtained by chance).

If two months are grouped together (*i.e.* sixty observations) then the correlation values having the same significance are 0.25 and 0.29. In other words, the same correlation on a larger number of observations becomes more significant, or less likely to be due to chance.

Variance is a measure of the variability of a factor and, mathematically, is the sum of the squares of the departures of the individual values from the mean. Thus in Table 11 the departures of the log. catch from the monthly mean on the first three days of June 1933 are -1.16 , -0.23 and -0.01 respectively. The total variance for the three days is, therefore, the sum of the squares of these values or $1.3456 + 0.0529 + 0.0001 = 1.3986$. Variance is always positive.

Covariance is a measure of the degree of interrelation of the simultaneous departures from the mean of two different variables, and is obtained by cross multiplying the two departures. On the first three days of June 1933 the departures from the monthly mean of the minimum temperatures were -5.0 , 0.7 and 0.6 . Thus from the figures already given above it will be seen that the covariance between catch and minimum temperature for the three days is

$$(-1.16 \times -5.0) + (-0.23 \times 0.7) + (-0.01 \times 0.6) = \\ 5.800 - 0.161 - 0.006 = +5.633.$$

Covariance is positive when the departures are both positive or both negative, and negative if the departures are of opposite signs.

These expressions, "regression" and "correlation," "variance" and "covariance," will be frequently used and it is important that a proper understanding of their meaning and limitations should be obtained.

When calculating a correlation or regression between the catch and a single arbitrarily selected factor of the environment, such as minimum temperature, it must be understood that the result obtained is really the effect on the catch of minimum temperature together with that of any other factor which is itself correlated with both catch and minimum temperature.

Thus it will be seen later that wind is positively correlated with minimum temperature and is negatively correlated with catch. Therefore if one makes a correlation or regression of minimum temperature with catch and neglects wind, one gets a value that will include the adverse wind effect and so will be smaller than the true temperature effect from which the wind has been eliminated. This problem will be taken up again later in the discussion of the simultaneous effect of two or more factors (see p. 288).

It should also be noted that the presence of a high correlation between two factors, or the possibility of calculating a significant regression, is in no way a proof that one factor is *directly* influencing the other. In the case above I have shown a significant correlation between the minimum temperature of the night and the number of insects caught. I infer from other knowledge that the probable explanation is that the higher night temperature is the cause of the high catch; but the purely mathematical process would lend just as much support to the idea that the large catch was the cause of the warm night! It is also possible, and frequently the true explanation, that the relation between the two correlated factors is not one of direct cause and effect, but that both are correlated with a third factor which has not appeared on the data provided.

Significant correlations and regressions, therefore, indicate a relationship,

but do not prove whether it is direct or indirect, or in which direction it is acting.

Two problems have next to be settled.

1. In what mathematical form are the catches to be expressed? Whether as actual numbers; as square roots; as logarithms; or by some other method?

2. Should the above values be used as they stand or should they be expressed as a departure from some fixed or changing mean, or as a difference between pairs of values? In this connection the possibilities are very numerous, but the following have been tested:—

(a) Values expressed as they stand.

(b) Values expressed as departures from a fixed monthly mean.

(c) Values expressed as departures from polynomial curves.

(d) Values expressed as departures from 31-, 29-, 15- and 5-day running means.

(e) Values expressed as differences between successive pairs of nights.

With reference to the first problem, I originally started calculations in this investigation using the numbers themselves, next I tried square roots, and finally abandoned these in favour of logarithms. The arguments for this have already been given (p. 234 and Williams 1936a and 1937). In the discussion below, therefore, logs will be used almost entirely.

It is, however, important to note that because the logarithmic transformation has been found the most suitable for the present problem, which deals

TABLE 11.

Catch, log. catch and departures of log. catch from various means in June 1933.

Date	Actual catch (n)	Log. catch log (n + 1)	Departures from means								Diff. between successive days	
			Monthly (fixed)		29-day (running)		15-day (running)		5-day (running)			
			+	-	+	-	+	-	+	-	+	-
1	15	1.20		1.16		1.30		1.10		0.57		0.93
2	134	2.13		0.23		0.37		0.14	0.20		0.22	
3	224	2.35		0.01		0.12	0.10		0.13		0.26	
4	406	2.61	0.25		0.20		0.37		0.16		0.22	
5	676	2.83	0.47		0.46		0.63		0.28			0.51
6	209	2.32		0.04		0	0.09			0.28	0.32	
7	431	2.64	0.28		0.36		0.33		0.11			0.06
8	379	2.58	0.22		0.33		0.16		0.12			0.32
9	187	2.27		0.09		0.02		0.19		0.10	0.23	
10	313	2.50	0.14		0.21		0.10		0.22			0.66
11	68	1.84		0.52		0.46		0.51	0.44		0.36	
12	159	2.20		0.16		0.09		0.08	0.25		0.40	
13	400	2.60	0.24		0.30		0.36		0.01		0.50	
14	1250	3.10	0.74		0.81		0.92		0.54		0.04	
15	1389	3.14	0.78		0.78		0.96		0.81			1.29
16	69	1.85		0.51		0.56		0.38	0.30		0.67	
17	14	1.18		1.18		1.30		1.13	0.71		0.31	
18	30	1.49		0.87		1.10		0.83	0.22		0.32	
19	63	1.81		0.55		0.72		0.57	0.03		0.41	
20	165	2.22		0.14		0.30		0.18	0.15		0.22	
21	98	2.00		0.36		0.53		0.28	0.10		0.65	
22	448	2.65	0.31		0.12		0.30		0.08		0.05	
23	396	2.60	0.24		0.07		0.32			0.09	0.56	
24	1460	3.16	0.80		0.62		0.75		0.36			0.31
25	714	2.85	0.49		0.34		0.30		0.04			0.10
26	563	2.76	0.39		0.21		0.05		0.14			0.06
27	493	2.69	0.33		0.15			0.09	0.01			1.08
28	40	1.61		0.75		0.92		1.17		0.94	1.72	
29	2119	3.33	0.97		0.87		0.48		0.63			0.97
30	229	2.36		0		0.05		0.48		0.50	1.16	
Variance (= sum of squares)			9.029		10.080		9.369		4.228		12.307	
Variance for June, 1935			33.456		12.449		7.369		4.513		9.396	

with varying catches in a light trap, it does not follow that it is suitable for other sets of figures. The main test of its suitability is that it reduces the frequency distribution curve of the data to an approximately normal and symmetrical form as already shown in fig. 3.

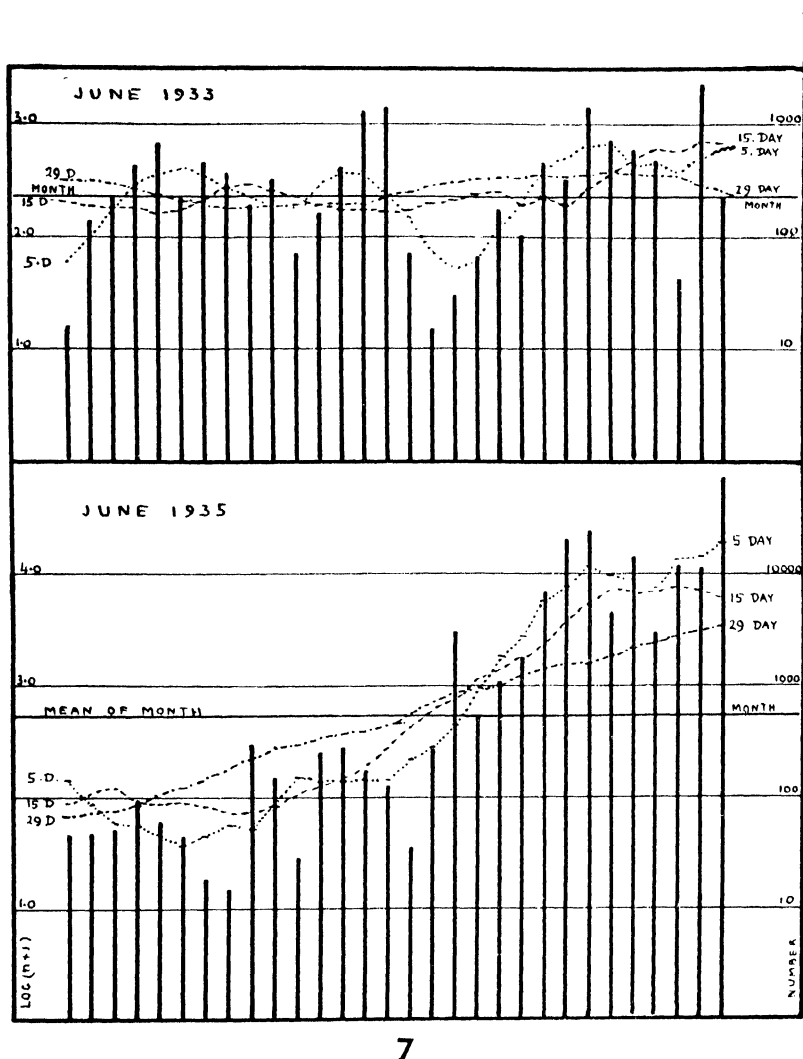
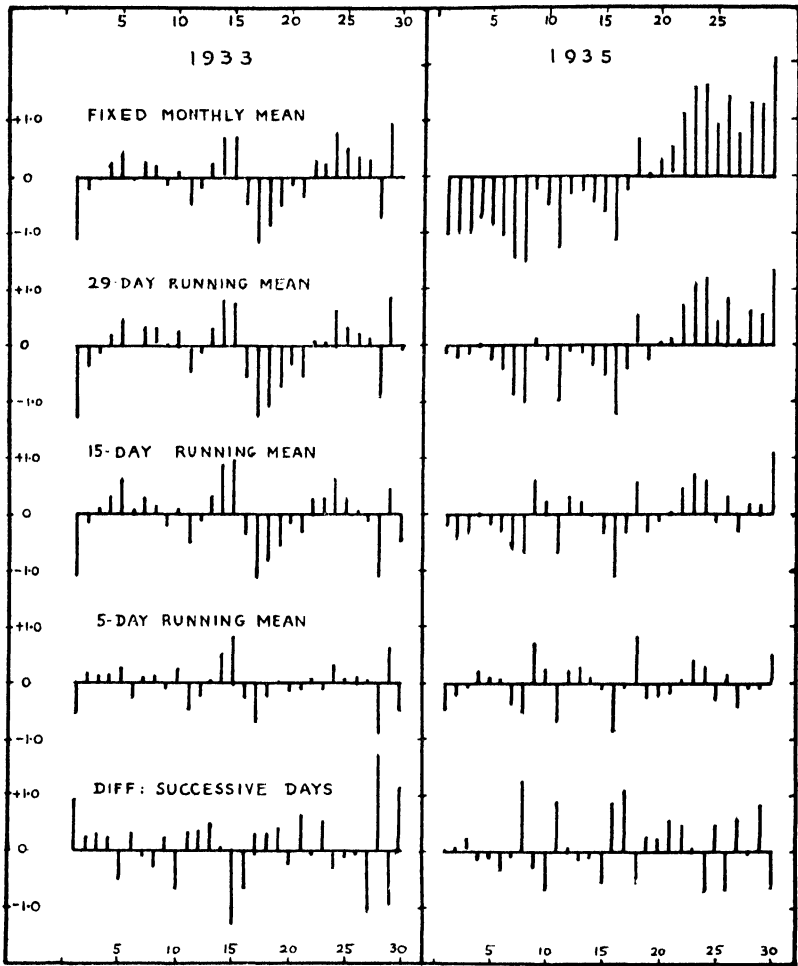


FIG. 7.—Histograms of log. catch of all insects in June 1933 and June 1935 with various means to indicate relation of mean to night's catch, and the effect on means of the definite trend in 1935.

Returning now to the question of the expression of catch and temperature as absolute values, as departures from fixed or running means, or as differences; some of the different possibilities of treatment for the same set of observations are shown in Table 11 and figs. 7 and 8. The table shows the value for the catch of insects each night in June 1933 expressed as a number (n) and as log.

($n + 1$); and the logs. are further shown as departures from a fixed monthly mean, from running means of 29, 15 and 5 days and as differences between successive days. It should be noted that the latter gives a value equal to twice the departure from a 2-day running mean.

Three points must here be emphasised. First, that whatever method is



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FIG. 8.—Departure of night's catch from various means in June 1933 and June 1935 to show methods of eliminating the trend of catch in 1935.

selected both variables must be treated by the same method. Thus if log. catch is expressed as departures from a 5-day mean, the minimum temperature must also be expressed in the same way.

Secondly, that the use of running means requires information of the values of catch and temperature outside the exact period to be dealt with. Thus the departures from a running 29-day mean for May can only be calculated if the

values for the second half of April and the first half of June are known. Thus more information is analysed with a long-period running mean than with a short-period running mean or with a fixed mean.

Thirdly departures from a fixed mean (*e.g.* monthly mean) give within a single month the same correlations and regressions as the actual values themselves over that period.

The total variation to be explained in each case (statistically speaking the "variance") is shown at the foot of Table 11. From this it may be seen that the variance in June 1933 is greatest for departures from fixed monthly means and from 29-day running means, and smallest for departures from 5-day means. If one divides the variance for the difference between successive days by 4 (since each difference is twice the departure from a 2-day running mean) one finds that this value is again smaller than the 5-day mean value. Thus the shorter the period of the running mean the less the variance. This will be seen also from fig. 7, which shows how the shorter period means follow more closely the variations in the catches.

The difference between the variance for the fixed monthly mean and the running mean depends largely on whether there is any definite trend in the values over the period under consideration.

In June 1933 there was no definite trend in the catch (see fig. 7) so that the variance from the fixed monthly means and from running 29-day means are almost the same. In June 1935 (see also fig. 7) there was on the contrary a very definite trend in the catch from low values at the beginning of the month to very high values at the end. This is immediately reflected in the variance as shown on the bottom line of Table 11. The variance from fixed monthly means is 33.456 in 1935 (*cf.* 9.029 in 1933) while that from the 29-day running mean is only 12.449 (*cf.* 10.080 in 1933).

The way in which the trend is eliminated by short-term means or successive differences is best seen in fig. 8, which shows graphically the departures from the mean for June 1933 (no trend) and June 1935 (very definite trend). The trend in 1935 is obvious in the monthly means and the 29-day running means, noticeable in the 15-day means (particularly at the beginning and end of the month) but is eliminated in the 5-day means and in successive differences.

Thus if fixed means are used for calculation the variance due to trends is included; if long-term running means are used variance due to steady trends is at least partly eliminated; if short-term running means are used variance due to both shorter and longer trends is eliminated. If there is a simultaneous trend in the same direction in both dependent and independent variables, the use of fixed means will tend to exaggerate both correlation and regression; if the trends are in the opposite direction the correlation and regression will be correspondingly diminished.

With short-term running means, however, much less of the total variation is discussed and there is a possible danger of omitting useful evidence by considering it as a trend rather than an immediate effect of the factor concerned. It is here that general knowledge of the problem has to be the deciding factor.

In the present case I believe that the long-period trends seen in the catch as it changes from month to month are largely a "total population" effect; while the rapid changes from night to night are much more definitely the effect of weather conditions in altering activity. If this is so, more accurate measurements of the effect of a factor (such as minimum temperature) on the *activity*

of insects will be obtained by the use of departures for a short-period running mean or from the difference between successive days.

One statistical difficulty, connected with the significance of the results, should, however, be pointed out here. If one uses the thirty catch values in one month as departures from a fixed- or long-period running mean one obtains a result the significance of which is based on 29 degrees of freedom. If, however, one takes the difference between successive days the fifteen values for the day differences 1-2; 3-4; 5-6 etc. are independent of each other and so are the values 2-3; 4-5; 6-7, but the two series are not independent. So that while the significance of the resulting regression can be calculated from either half set of differences (each with 14 degrees of freedom), the result based on all the possible differences, although more accurate than either half set, cannot be considered as if it were based on twice as many degrees of freedom.

Polynomial means of the third and fourth degrees were calculated for the catch and minimum temperatures for June 1933, but only based on the square root of the catch, and not on the log. No advantage seemed to be gained by their use and they suffered from several practical disadvantages as follows: firstly the long time necessary for the calculation; secondly the shape of the polynomial curve near each end depends on how many values were taken on either side of the series; and thirdly polynomial curves calculated separately for two successive months do not "join up," so that the mean on the last day of one month is quite different from that of the first day of the next month. For these reasons no further calculations were made on this basis.

TABLE 12.

Correlations and regressions between log. catch and minimum temperature for the same months calculated from different means.

	June				July	
	1933		1935		1933	
	Cor.	Reg.	Cor.	Reg.	Cor.	Reg.
Departures from—						
Monthly mean	0.73	0.123	0.77	0.161	0.29	0.068
29-day running mean . .	0.61	0.091	0.52	0.094	0.34	0.075
15-day running mean . .	0.74	0.110	0.44	0.062	0.34	0.064
5-day running mean . . .	0.59	0.107	0.37	0.051	0.15	0.021
3-day running mean . . .	0.65	0.109	0.25	0.043	—	—
Difference between successive days	0.71	0.100	0.36	0.051	0.24	0.025

Regressions and correlations have not been calculated by all these methods for all the 48 months; they have, however, all been done for the three months June and July 1933 and July 1935, and in addition calculations have been made from a running 3-day mean. These are all shown in Table 12.

The effects of the different methods in each month vary considerably and are difficult to understand. Some of them are accidental, due to the relatively small number of observations. It is, however, easy to see, in June 1935, the effect of the simultaneous trend from low to high values in both catch and minimum temperature, which causes an abnormally high regression when departures from a fixed monthly mean are used.

The essential differences between the use of departures from a fixed monthly mean as one extreme, and the differences between successive days as the other,

in the calculation of correlations and regressions is shown in Tables 13 and 14 and in fig. 9.

Table 13 shows the regressions and correlations of log. catch and minimum temperature calculated from departures from monthly means for each of the 48 months separately; for the four repetitions of each month; for each of the four years; and for the four years together.

TABLE 13.

Correlations and regressions of log. catch on minimum temperatures from departures from monthly means.

	1933-34		1934-35		1935-36		1936-37		All 4 years	
	Cor.	Reg.	Cor.	Reg.	Cor.	Reg.	Cor.	Reg.	Cor.	Reg.
March .	0.43	0.052	0.45	0.056	0.65	0.098	0.53	0.064	0.54	0.070
April .	0.57	0.063	0.34	0.031	0.38	0.048	0.78	0.100	0.52	0.058
May .	0.43	0.111	0.75	0.107	0.70	0.106	0.69	0.096	0.65	0.105
June .	0.76	0.122	0.58	0.071	0.77	0.161	0.85	0.120	0.74	0.124
July .	0.21	0.061	0.42	0.043	0.62	0.083	0.17	0.030	0.39	0.060
Aug. .	0.68	0.084	0.74	0.082	0.79	0.105	0.31	0.056	0.63	0.084
Sept. .	0.42	0.048	0.77	0.083	0.22	0.023	0.59	0.062	0.52	0.056
Oct. .	0.70	0.093	0.73	0.070	0.56	0.065	0.58	0.085	0.63	0.077
Nov. .	0.43	0.078	0.74	0.110	0.39	0.059	0.37	0.053	0.50	0.077
Dec. .	0.39	0.072	0.51	0.073	0.61	0.055	0.45	0.058	0.50	0.060
Jan. .	0.53	0.060	0.54	0.103	0.63	0.070	0.58	0.089	0.50	0.080
Feb. .	0.04	0.005	0.28	0.036	0.53	0.063	0.64	0.118	0.44	0.064
Year .	0.50	0.070	0.52	0.074	0.57	0.075	0.55	0.078	0.56	0.074 0.0030

TABLE 14.

Correlations and regressions of log. catch on minimum temperature from differences between successive days.

Period	Cor.	Reg.	Period	Cor.	Reg.
Four March . . .	0.37	0.069	Four Springs . . .	0.39	0.058
April	0.32	0.042	Summers	0.45	0.063
May	0.45	0.074	Autumns	0.41	0.056
June	0.54	0.080	Winters	0.38	0.056
July	0.33	0.044			
August	0.50	0.063	1933-34	0.38	0.054
September	0.47	0.045	1934-35	0.39	0.053
October	0.51	0.066	1935-36	0.41	0.062
November	0.29	0.056	1936-37 (10 months)	0.43	0.073
December	0.33	0.060			
January	0.39	0.063	All four years	0.40	0.060
February	0.44	0.079			0.0037

Table 14, on the other hand, shows the corresponding values for the four repetitions of each month, for each year, and for the four years together, calculated from the difference between successive days.

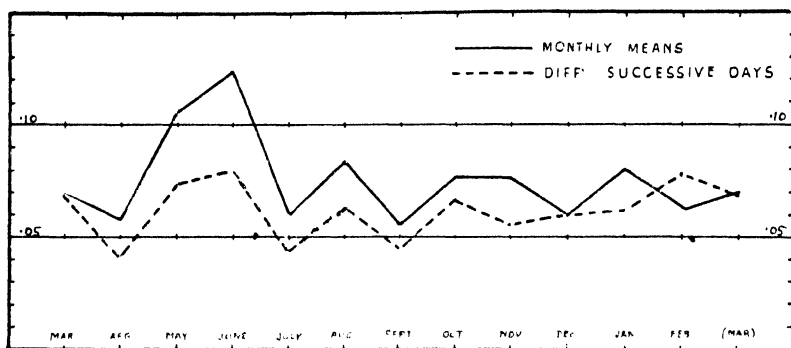
The average monthly regression calculated by each method is shown diagrammatically in fig. 9.

Two main differences are apparent. First that the regression by the method of differences between successive days is generally smaller than the value from

fixed monthly means. Secondly the values by the latter method for May and June are very much higher than the rest.

I think that both these differences are explained by the reasoning already put forward; namely, that the use of the fixed monthly mean causes the results of simultaneous trend to be included in the regression and correlation. In May and June both catch and temperature are increasing, and give a falsely high value to the regression, if this is looked upon only as a measure of "activity." In the autumn the difference is not so well marked, as the normal fall in the insect population is masked by the arrival in the trap of great numbers of winter gnats (*Trichocera*). This causes the average log. catch to remain high, and the average catch for November to be as high as that for October.

The mean regression of log. catch (of all insects) on minimum temperature for the whole period of four years, if calculated by the departure from fixed monthly means, is 0.074 ± 0.0030 per degree Fahrenheit, and the correlation 0.56 (on over 1400 values). This means that the catch is doubled (i.e. log.



9

FIG. 9.—Regression of log. catch of all insects on minimum temperature. Mean of each month in all four years, calculated by departures from monthly means (solid line) and by differences between successive days (dotted line).

raised by 0.301) by an increase of temperature of 4° F. (2.2° C.); or, in the usual physical notation, Q_{10} (the increase over 10° C.) = 21.5.

With the calculation based on differences between successive days the regression is 0.060 ± 0.0037 or the catch is doubled by 5° F. (2.8° C.); giving $Q_{10} = 12.8$. If one is discussing "activity" only, this latter is almost certainly the more correct estimate.

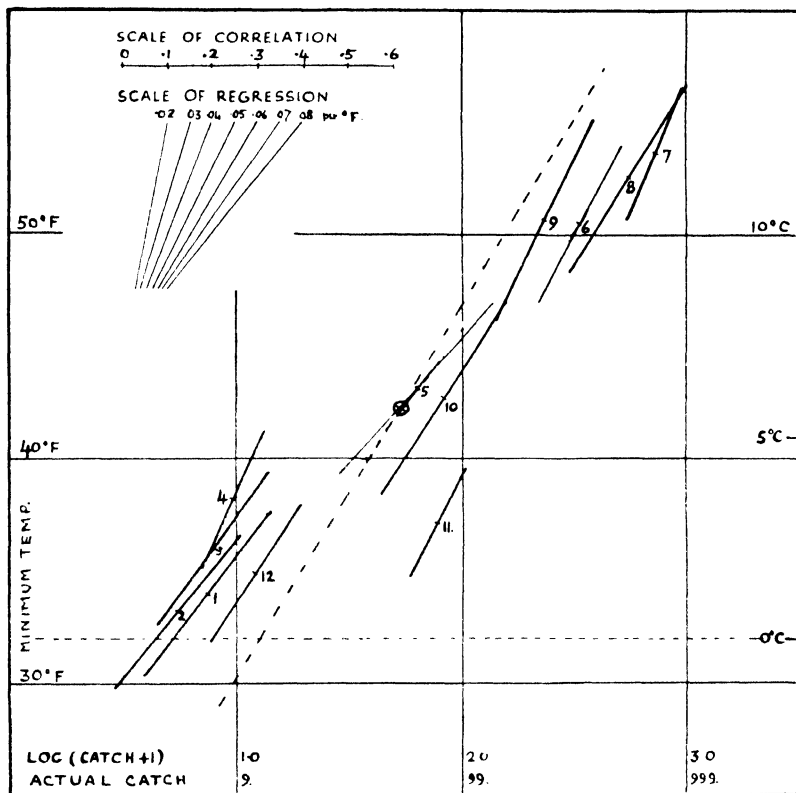
The values for the average regressions of catch on minimum temperature for each month in the year, based on the difference between successive days, are shown diagrammatically in fig. 10, together with the mean value for all four years as a dotted line. In this diagram an attempt has been made to indicate simultaneously both regression and correlation by making the length of the regression line proportional to the correlation according to a scale indicated on the diagram. Thus the longer the regression line on the figure the higher is the correlation.

Figs. 9 and 10 show quite definitely that there is practically no evidence of any seasonal change in regression value. The values for each month vary round the mean without any regular sequence.

As further evidence on this question an analysis of variance has been cal-

culated on the regressions (by method of differences) for the 48 months, showing the mean variance between months and between years as follows :—

	Degrees of freedom	Total variance	Mean variance
Total	47	38,887	827
Between years	3	2,316	772
Between months	11	7,080	644
Error	33	29,491	894



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FIG. 10.—Diagrammatic representation of regressions and correlations between log. total catch and minimum temperature in the different months of the year (each the average of 4 years) and the mean for the whole 48 months (dotted line). The length of the regression line represents the correlation on the given scale. The months of the year are represented by the numbers 1-12. Results calculated from differences between successive days.

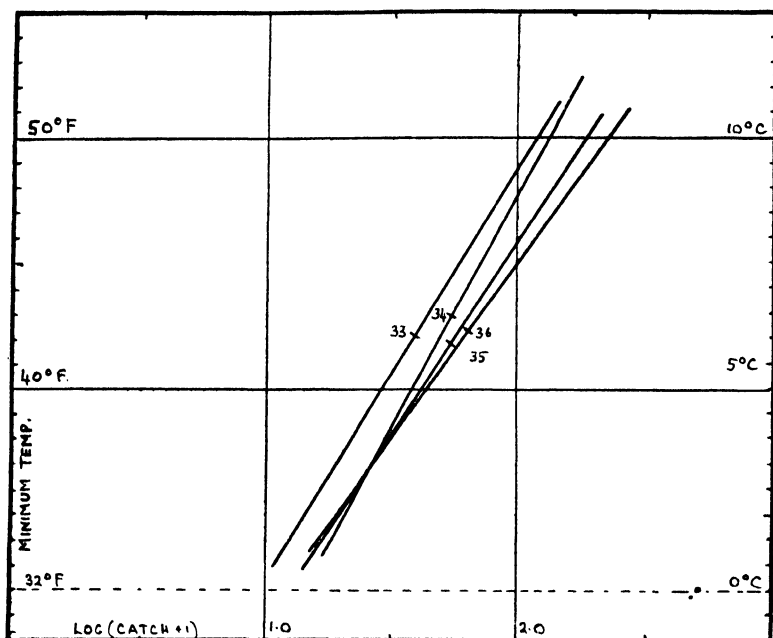
From this it will be seen that the mean variance between months and the mean variance between years are both less than the mean variance or the mean error, thus giving no evidence of either seasonal or annual variation.

If this interpretation is correct, and since the insect population caught in the trap is composed of quite different species in summer and winter, it would appear that the reaction of insect activity to minimum night temperature is not, on the average, specific to different insects, but is a fundamental property

of large groups and divisions of insects. In other words, the insects found at Rothamsted in the summer are reacting to minimum temperature changes in a very similar way to those found in winter although the species and often the families and orders are quite different. This will be referred to again later when the regressions for different groups of insects are discussed separately (see p. 263).

Fig. 11 shows diagrammatically the regression lines of the four successive years in which the trap was run, based on the difference between successive days. The similarity of the results is particularly striking.

To sum up, the method of calculating regressions and correlations from



II

FIG. 11.—The regression lines for log. all insects and minimum temperature for each of the four years of trapping. Calculated from differences between successive days.

the differences between successive days appears to be the most reliable one, and when this is done it is found that the effect of the raising of the night minimum temperature by 1° F. is to raise the log. catch by 0.060 ± 0.0037 . Otherwise the catch is doubled by a rise in temperature of just under 5° F., or increased 13 times by a rise of 10° C.

The effect of minimum temperature on other than "Total Catch."

(a) *Insects in last period of night only.*

As the minimum temperature occurs usually just before dawn in the last period of the night, a calculation was made for the three months June to August 1933 to see if there was a closer relation between the minimum temperature

and the catch in the last period, than with the total catch for the whole night. The results are shown in Table 15.

It will be seen that there are slight differences within a single month; thus the correlations are lower in two months and the regression lower in one month for the 8th period only; but when the three months are combined there is no significant difference in either correlation or regression.

TABLE 15.

Correlations and regressions of log. catch in the last period of the night on minimum temperature.

1933	Correlation		Regression	
	8th period	Whole night	8th period	Whole night
June	0.54	0.73	0.109	0.123
July	0.34	0.29	0.069	0.068
August	0.65	0.68	0.083	0.084
Three months	0.53	0.56	0.088	0.091

(b) *Single orders of insects.*

It has been shown above that when the regression on minimum temperature for all insects is calculated for each month of the year there is no significant difference between the values for the different months. But in the winter the catch is almost entirely Diptera while in July nearly 25% of the catch are Lepidoptera and other groups. Also the species of Diptera caught in the winter are different from those in the summer. It thus appeared that the regression might be typical of all insects and not vary from order to order or from species to species.

To test this in more detail, regressions on minimum temperature have been calculated for all Lepidoptera for the 3 summer months, June, July and August in the four years, and also for all Hemiptera for the two Augusts (1933 and 1935) in which over 1000 individuals were captured per month. In August of the other two years the numbers captured were too small to give reliable regressions.

The figures so obtained are shown in Table 16 with the corresponding

TABLE 16.

Correlations and regressions of the catch of Lepidoptera and of Diptera on minimum temperature.

	Cor.	Reg.	All insects in same period for comparison	
All Lepidoptera—				
3 June, 1933–35 ² . . .	0.60	0.0795	0.53	0.0799
4 July, 1933–36 . . .	0.36	0.0406	0.33	0.0442
4 August, 1933–36 . . .	0.55	0.046	0.49	0.0632
Total 11 summer months . . .	0.48	0.0516 \pm 0.0052	0.44	0.0597 \pm 0.0065
All Hemiptera—				
2 August, 1933 and 1935 . . .	0.61	0.083 \pm 0.014	0.60	0.076

² In June 1936 the trap was not working regularly.

values for "all insects" for the same period. It will be seen that in no case are the differences significant. In the case of the Lepidoptera for the total of eleven months the difference between the two regressions is 0.0082 and the standard error of the difference 0.0084, so that this result could have been obtained by chance in 30% of trials.

In the Hemiptera for the two Augusts the difference is actually smaller and the error greater.

The question must finally be tested by a study of single species but this problem will be dealt with in a later publication.

A calculation was also made of the correlations and regressions of the moths of the family NOCTUIDAE in the three summers (May to October) of the three years 1933-35. The figures for the NOCTUIDAE on which these are based had already been corrected for lunar effect by a method described in a previous paper (Williams 1936a). The results are shown in Table 17.

It will be seen that on the whole eighteen lunar months (526 days) the effect of one degree change in minimum temperature is to alter the log. catch by 0.051 ± 0.005 , which is quite comparable with the results obtained for all insects and for Lepidoptera only.

TABLE 17.

Correlations and regressions of the catch of NOCTUIDAE (corrected for lunar effect) and minimum temperature; from departures from monthly means.

Lunar month	1933		1934		1935	
	Cor.	Reg.	Cor.	Reg.	Cor.	Reg.
May	0.29	0.050	0.40	0.052	0.18	0.016
June	0.51	0.078	0.37	0.058	0.53	0.099
July	0.19	0.031	0.23	0.029	0.22	0.031
August	0.36	0.062	0.28	0.039	0.67	0.071
September	0.26	0.033	0.44	0.036	0.18	0.024
October	0.74	0.113	0.69	0.057	0.42	0.047
Six months	0.42	0.068	0.39	0.045	0.35	0.043
All 3 years			0.39	0.051 ± 0.005		

The error of the regressions on minimum temperature.

It has not been considered necessary to work out the standard error of the regression in all cases, but this has been done in several which are summarised in Table 18. Theoretically the error of the regression is inversely proportional to the square root of the number of observations. Thus a calculation based on twelve months should be twice as accurate as one based on only three months' observations. It will be seen how closely the calculated errors conform to this relation; and in the last column an approximate theoretical value has been given which can be used to find the length of the period of observation necessary to give any required accuracy.

Maximum temperature of the previous day.

Table 19 shows a summary of the correlations and regressions for total catch and maximum temperature calculated from the differences between successive days.

It must be remembered that this calculation, being a single regression, includes the positive or negative effect of any associated factors.

TABLE 18.

Errors of regressions on minimum temperatures in calculations for different numbers of months.

Period	Insects	Date	Error of regression	Approx. value by theory
One month	All insects	June 1933	0.021	} 0.024
Two months	"	June 1934	0.024	
Four months	All Hemiptera	Aug. 1933 and 1935	0.014	} 0.014
	All insects	Four Julys	0.012	
		Four Septembers	0.0077	} 0.012
Eleven months	All insects	Four Summers	0.0065	
	All Lepidoptera	"	0.0052	} 0.0072
Four years	All insects	(Partial reg.)	0.0037	
	"		0.0038	} 0.0035

It will be seen that the final regression for all four years is 0.042 (as compared with 0.060 for minimum temperature), which indicates that the catch is doubled following a rise of maximum temperature of about 7° F.

In the series of regressions of the individual months the lowest regression is in March (with a very low correlation) and the highest in December with a high correlation, but it is not really certain that the differences between these extremes are significant, particularly as the month following March gives a regression exactly equal to the average.

Spring gives a regression a little below the normal and winter a little above but again it is doubtful if their differences are significant.

It is curious to note that the year 1936-37 gives a very much higher regression than the other years and almost double that of 1933-34. The difference is mathematically significant on the error of regression. It will be seen from Table 2 (p. 231) that in 1933-34 the maximum temperatures were above the average of the four years in all the months from March to October. While in 1936-37 they were below the average except in May and December to February. This gives, therefore, a suggestion that maximum temperatures may have a greater importance in years of low maxima than in years of high maxima.

TABLE 19.

Correlations and regressions for log. catch on maximum temperature of the previous day; from differences between successive days.

Period	Cor.	Reg.	Period	Cor.	Reg.
Four March .	0.03	0.005	Four Springs .	0.21	0.032
April .	0.32	0.042	Summers .	0.34	0.043 \pm 0.007
May .	0.28	0.038	Autumns .	0.20	0.044
June .	0.37	0.044	Winters .	0.25	0.050 \pm 0.011
July .	0.30	0.039 \pm 0.012			
August .	0.36	0.045	1933-34 .	0.19	0.030 \pm 0.008
September .	0.29	0.043	1934-35 .	0.27	0.041
October .	0.28	0.063	1935-36 .	0.19	0.036
November .	0.08	0.022	1936-37 .	0.33	0.065 \pm 0.011
December .	0.35	0.091			
January .	0.30	0.055 \pm 0.016	All four years .	0.24	0.042 \pm 0.005
February .	0.10	0.017			

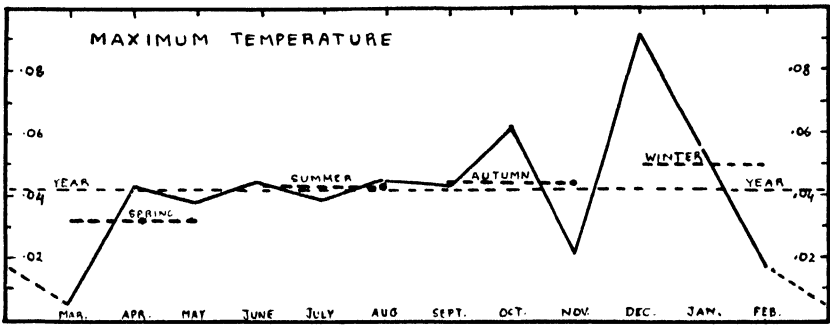
Fig. 12 shows the above results graphically for comparison with the similar figure (9) for the minimum temperature. It will be seen that the regressions are very even from April to September, but very irregular during the late autumn and winter months from October to March. There is no real evidence of regular seasonal change.

Analysis of variance of the regression for the 48 months shows, as in the case of the minimum temperature, that the differences between months and between years are both smaller than the mean error.

Grass minimum temperature.

Grass minimum temperature is closely correlated with the screen minimum temperature, but is considerably more variable. For example, the total variance (sum of squares) for the six months (178 days) June, July and August in 1933 and 1934 is 7252 for the grass minimum and only 3471 for the screen minimum.

Table 20 shows the regressions and correlations calculated by the method



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FIG. 12.—Sequence of mean monthly regressions of log. total catch on the maximum temperature of the previous day, calculated from the difference between successive days.

of differences between successive days for each of these six months, and also the total for each summer and the two summers combined. It will be seen that the

TABLE 20.

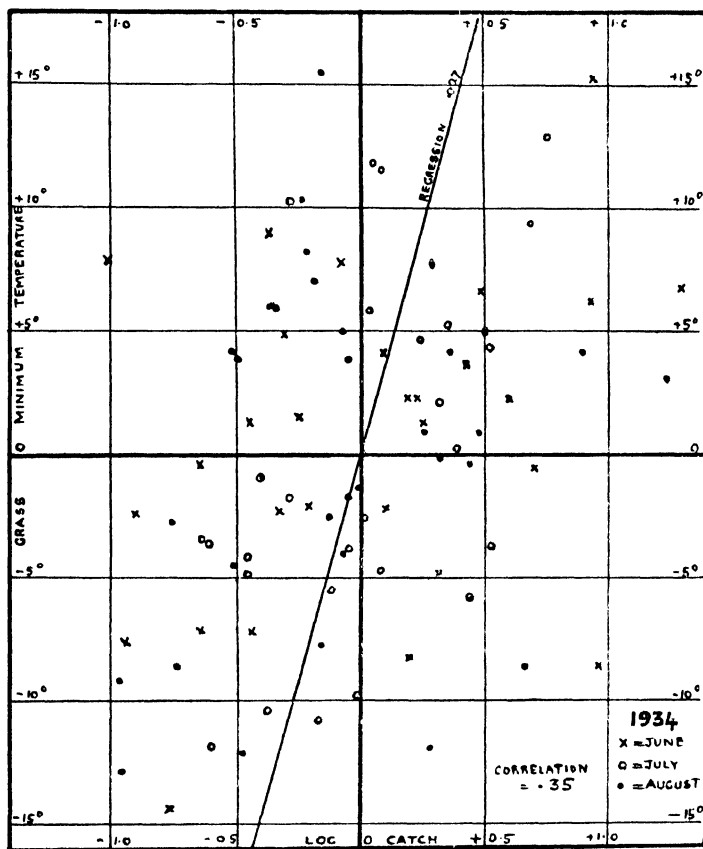
Correlations and regressions for log. catch and the grass minimum temperature, from differences between successive days.

	1933		1934	
	Cor.	Reg.	Cor.	Reg.
June	0.64	0.074	0.35	0.0333
July	—0.04	—0.003	0.50	0.0278
August	0.41	0.036	0.26	0.0203
All 3 months	0.28	0.0339	0.35	0.0268
All six months		Cor.		Reg.
Figures of screen min. for comparison		0.35		0.0299
		0.48		0.0583

final correlation is 0.35 and that each degree change of grass minimum temperature alters the log. catch by 0.030. Thus a rise of approximately 10° F. will double the catch.

In the same table it is shown that the corresponding figures for the same period for the screen minimum are 0.48 and 0.058; both considerably higher.

Analysis of variance shows a mean daily variance of 0.2921 for the trap for



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FIG. 13.—Scatter diagram and regression lines for the relation between the log. total catch and the grass minimum temperature for June, July and August 1934 from difference between successive days.

the six months, of which only 0.0337 (12%) is explained by the regression on the grass minimum and 0.0651 (22%) by the regression on screen minimum. The screen minimum therefore is a better indication of the catch variation than is the grass minimum.

Fig. 13 shows as a scatter diagram the catch and grass minimum on all nights in the summer of 1934, expressed as differences between successive days; and also the regression line through these points.

Daily range of temperature.

The discussion on p. 246 on the differences in daily range between nights of high and low catch made it unlikely that any significant relation would be found. However, to bring the data in a line with the other analyses, regressions and correlations have been calculated for the three summer months in 1933, by the method of differences between successive days, and are shown in Table 21.

TABLE 21.

The relation of log. catch to daily range of temperatures from differences between successive days.

	Cor.	Reg.
1933 June	-0.32	-0.039
July	+0.05	+0.003
August	+0.10	+0.011
All three months	-0.05	-0.0039

The results show that in one month the relation was negative and in the other two months positive, and that the average of the three months is a small negative effect which is well below the limits of significance. The scatter diagram for all these months is given in fig. 14, and shows graphically the absence of any definite relationship.

Humidity.

Owing to the close manner in which air humidity is tied up with temperature very little analysis was made on the direct effect of this factor on catch.

On p. 249 has already been given a comparison of the relative humidities on nights of high and of low catch and later there will be given simultaneous analyses of humidity and 9 p.m. temperatures (see p. 291); and humidity wind and maximum and minimum temperature (294).

Table 22, however, shows the simple correlation and regression of 9 p.m. relative humidity on total catch in the year 1933-34. The other years cannot be added as the 9 p.m. observations were discontinued and the recording instrument was not sufficiently accurate.

It will be seen that spring, autumn and winter all give positive correlations and regressions, of which the autumn values are the highest. The summer value is a non-significant negative one. For the whole year the regression is $+0.0078 \pm 0.0037$, which is just significantly positive at the 5% level but not at the 2%.

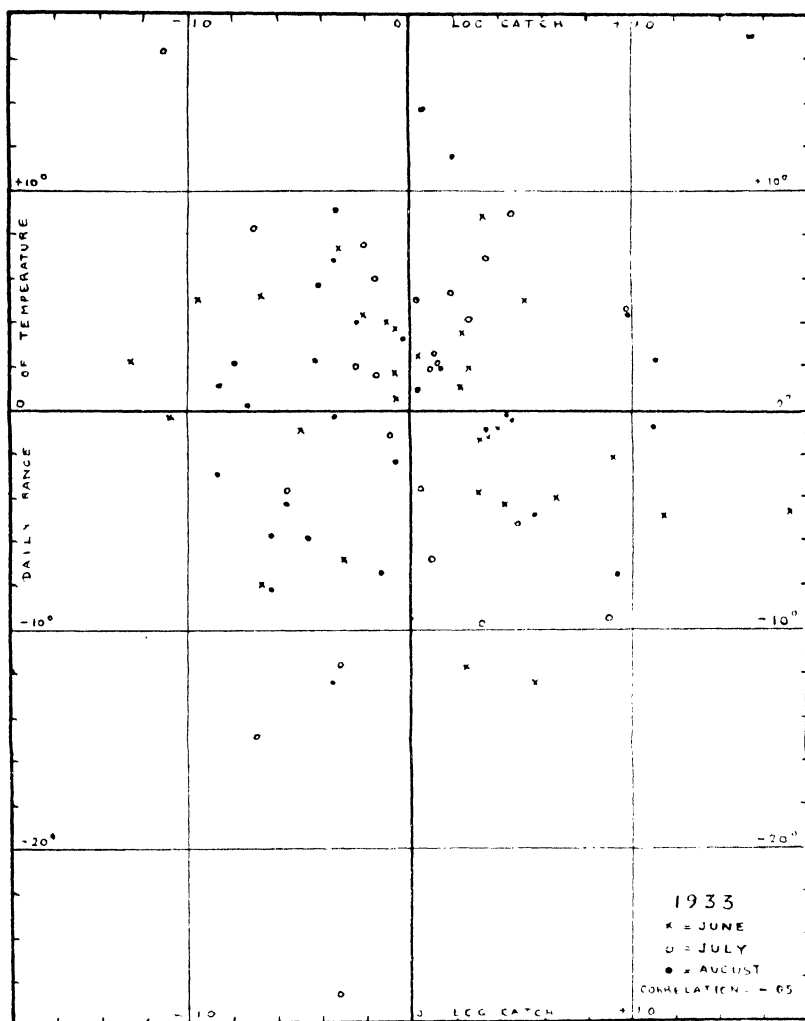
TABLE 22.

Correlations and regressions of insects on relative humidity at 9 p.m.; from differences between successive days, 1933-34.

	Cor.	Reg.
Spring (March-May)	+0.14	+0.0093
Summer (June-August)	-0.03	-0.0013
Autumn (September-November)	+0.22	+0.0193
Winter (December-February)	+0.16	+0.0138
All year	+0.11	+0.0078 \pm 0.0037

Rain.

Owing to the evidence already produced (p. 247) that there is practically no difference between the incidence of night rain between nights of high and low catch, little statistical analysis has been done.



14

FIG. 14.—Scatter diagram of relation between log. total catch and daily range of temperature for June to August 1933, showing the absence of any correlation between the two.

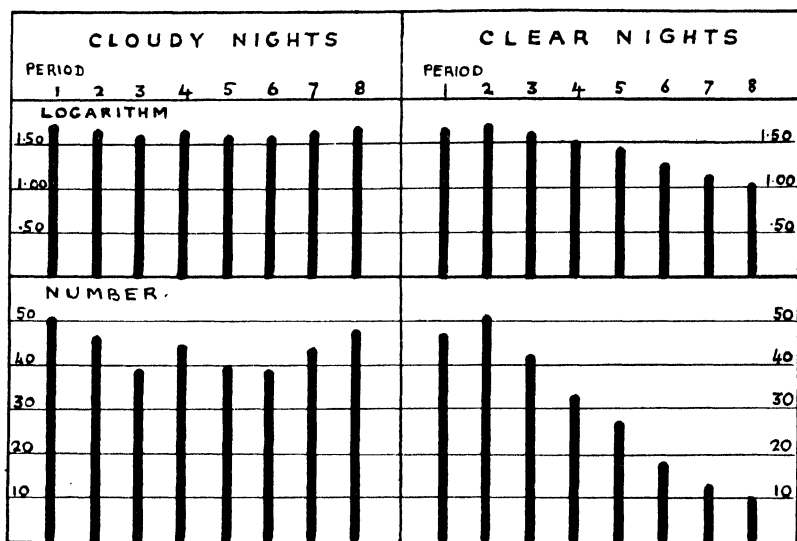
However, a study was made of the 66 nights in 1933, on which rain fell during the period 6 p.m. to 6 a.m., and this showed that these nights had a log. catch 0.02 above normal, and that the regression was $+0.017$ per 0.1 inch of rain. On further inspection, however, it was found that the rainy nights

had an average minimum temperature 1.3° F. above normal, which is in itself more than sufficient to account for the slight increase in catch.

On the other hand, an analysis of the effect of day rain (in the period 6 a.m. to 6 p.m.) over the same period showed that the 71 nights following day rain had an average log. catch $+0.168$ below normal, and that the regression was -0.068 per 0.1 inch of rain. In this case, however, it is found that the average temperature of the nights is 0.7° F. below normal, so part at least of this effect of day rain may be caused by the lowered temperature.

Cloud.

No direct calculations have been made on the effect of cloud on the total number of insects captured during the night as this effect is mostly indirect.



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FIG. 15.—Distribution during the eight periods of the night of all insects on clear and on cloudy nights. Given first on logarithmic basis and then as actual number.

The amount of cloud affects night temperature, humidity and moonlight, and through them the catch.

In the course of the work, however, a comparison has been made of the distribution of the insects in the eight periods of the night into which the catch is divided (see Williams 1939 : 119) on the 46 cloudy nights and the 52 clear nights in the summer of 1935, from May to October inclusive.

The figures are shown in Table 23 and diagrammatically in fig. 15. It will be seen how much later the night activity continues when the sky is covered with clouds. In fact the number of insects at dawn on the cloudy nights is almost as great as at dusk, whereas on the clear nights the catches at dawn are only about one-fifth of those at dusk.

Other details on the distribution of insects will be found in the first part of this paper (Williams 1939).

The combined effect of cloud and moon is discussed on p. 274.

TABLE 23.

Difference between night distribution of insects on clear and cloudy nights during the summer of 1935.

	Period of night							
	1	2	3	4	5	6	7	8
As log. ($n + 1$):								
Clear	1.68	1.72	1.63	1.53	1.45	1.27	1.13	1.06
Cloudy	1.72	1.68	1.60	1.66	1.61	1.60	1.65	1.69
As number (antilog. of above -1):								
Clear	47	51	42	33	27	18	13	10
Cloudy	51	47	39	45	40	39	44	48

Fog.

Fog is of course only an extreme case of high humidity, super-saturation or a negative "saturation deficiency." It has, however, a quite new biological effect owing to its interference with visibility.

In general in the Rothamsted district fog occurs during the late autumn and winter months, especially in November and December, and on nights when the temperature is below normal. The catches on such nights are usually very poor.

In 1933, however, on the night of the 28th September there was a dense fog, starting about 4 p.m. and lasting throughout the night and the early part of the following day; visibility was at times not more than a few yards. The catches, particularly in the family NOCTUIDAE, on that night were so unusual that they seem worthy of special discussion.

The maximum temperature on the 28th September was 67.3° F. (exactly normal for the month but a little above the average for the end of the month). The minimum temperature was 45.4° F. (6.1° F. below the month's normal) and the grass minimum 41.4° F. (5.1° F. below the month's normal). Two traps were working on that night: one, trap A, in its usual position; the other, trap B, on the roof of the Entomological Laboratory at a height of about 35 feet. Both had the same light intensity (see Williams 1939: 93 for general discussion of the catches in this trap).

The capture of all insects for trap A was 148, of which 37 were Lepidoptera and 100 Diptera. The mean catch per night for the month in this trap was 221 so that the foggy night gave a total below the average. The Lepidoptera, however, included 36 NOCTUIDAE of 5 species, which was the second highest total of this family for the month and far above the month's average of 12 NOCTUIDAE per night. The distribution of the NOCTUIDAE in the successive periods of the night was 0: 0: 8: 9: 4: 13: 2: 0.

In trap B only the Lepidoptera were recorded, and these included 104 NOCTUIDAE of 14 species, again the second highest total of the month and more species than on any other night in the month.

The actual species captured are shown in Table 24.

All the species in trap B, except *A. lychnidis* and *A. lunosa*, came in larger numbers than on any other day in the month. Twelve *P. meticulosa* were captured in trap B out of a total of only 37 captured in both traps in all four years.

The most remarkable case is, however, that of *A. circellaris*. Twenty-two

TABLE 24.

Captures of NOCTUIDAE on night of heavy fog in September 1933.

	Trap A	Trap B
<i>T. pronuba</i> (L.)	—	2
<i>A. ypsilon</i> (Rott.)	—	5
<i>A. litura</i> (L.)	—	4
<i>P. flavicincta</i> (S.V.)	—	5
<i>A. lychnidis</i> (Rbr.)	30	34
<i>M. gilvago</i> (Esp.)	3	4
<i>A. lunosa</i> (Hw.)	1	2
<i>P. gamma</i> (L.)	—	2
<i>A. lutulenta</i> (Bkh.)	—	4
<i>A. saucia</i> (Hübner)	—	3
<i>P. meticulosa</i> (L.)	—	12
<i>E. satellitia</i> (L.)	—	2
<i>A. circellaris</i> (Hufn.)	1	22
<i>A. segetum</i> (Schiff.)	—	3
<i>N. c-nigrum</i> (L.)	1	—
Total	36	104

of this species were captured in trap B and one in trap A. Throughout the whole of the remaining nights in which either of the two traps were used, only one other individual was captured in trap A (in 1934) and four others in trap B (2 in 1933 and 2 in 1934); so that out of a total catch of 28 in both traps in four years (trap B not in continuous use) 23 came on this one night of thick fog.

It is difficult to suggest an explanation, particularly as no other fog occurred so early in the autumn in the other three years. It is, however, possible that some of the insects found the broad area of illuminated fog more attractive than the bright point of light; or again it is possible that the fog did not extend very high above the ground and that many moths were flying above it and were attracted to the upper light. This would not, however, explain the catch of NOCTUIDAE in the lower trap (only 3 feet 6 inches above the ground), which was larger than normal, though not so large as that in the upper trap.

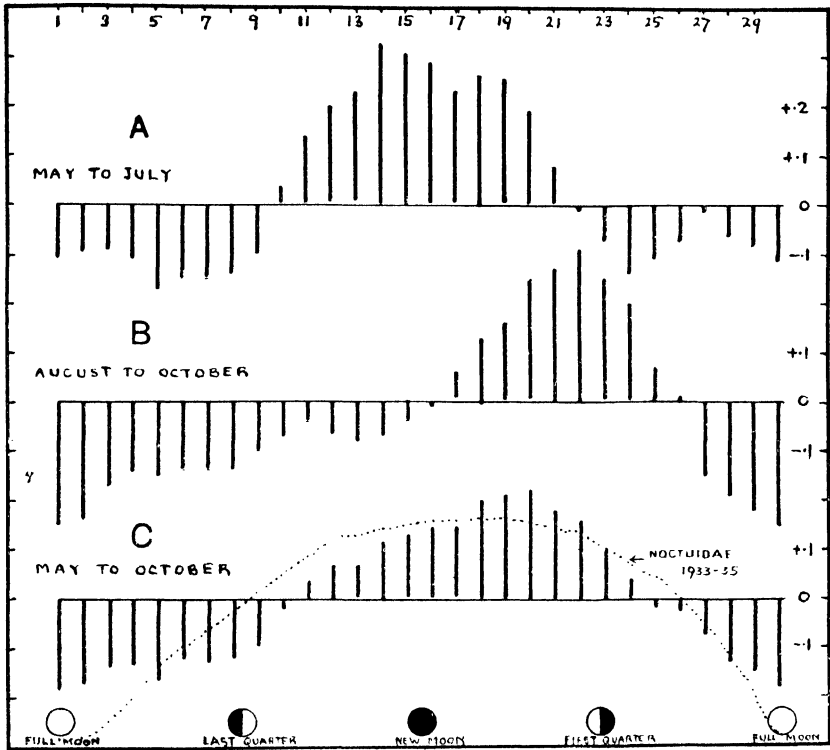
Moonlight.

In a previous paper (Williams 1936a) I have discussed in some detail the effect of moonlight on the catches in the trap, particularly on the family NOCTUIDAE of the Lepidoptera. Tables and figures were then given showing the average departure of the log. catch from the mean on each successive day of the lunar period in the summer months of 1933-35.

It is now possible to add another year (1936) to the analysis, but it has been considered more interesting to deal this time with "all insects" instead of with the NOCTUIDAE only. The results for all four years for the six summer months are shown in Table 25 and also, smoothed to a 5-day running mean in fig. 16, C and as actual numbers (with the mean catch 100) in fig. 17. It will be seen that the maximum negative departure in the log on the smoothed curve is -0.18 on the day of full moon (equivalent to a catch 34% below normal) and the maximum positive departure is +0.22 a few days after new moon (equivalent to a catch 66% above normal). The dotted line superimposed on the histogram shows the results previously obtained for the NOCTUIDAE

in the years 1933-35. The insects as a whole show a less extreme negative departure at full moon than the NOCTUIDAE, but a higher positive departure in the first quarter. The range in percentage of the mean is, as seen in fig. 17, from 66% at full moon to 166% during the first quarter.

It will be noted that the curve is slightly asymmetrical. It was suggested in the previous paper (Williams 1936a) that this was due to certain asymmetries in the time of rising and setting of the moon. These are different before and



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FIG. 16.—Diagram showing the average log departure from the mean of the catches of all insects on the successive days of the lunar month. Each shows the average of the six summer months in all four years and they are smoothed to 5-day running means. The dotted line in the lower figure shows the results previously obtained for the family NOCTUIDAE for the three years 1933-35.

after the middle of June, and it was shown that if the results obtained for the NOCTUIDAE are calculated in two portions, one before and one after the middle of June, they show opposite asymmetries as expected by the theory. The asymmetry of the full curve May to October is thus merely due to the fact that it contains a longer period with one asymmetry than the other.

An attempt has been made to demonstrate the truth of this relationship from the data now available for all insects. This has been divided into two series: the first including May, June, and July and the second August, September and October (Table 25 and fig. 16, A and B). If the reasoning is correct the

TABLE 25.

Departure of log. catch of all insects from the normal on successive days in the lunar months in May to October of the four years.

Day of lunar month	May to July		August to October		May to October	
	Actual	5-d. mean	Actual	5-d. mean	Actual	5-d. mean
(Full) 1	-0.21	-0.11	-0.31	-0.25	-0.26	-0.18
2	-0.11	-0.10	-0.26	-0.24	-0.17	-0.17
3	-0.16	-0.09	-0.32	-0.17	-0.24	-0.13
4	+0.08	-0.11	-0.02	-0.14	+0.02	-0.13
5	-0.07	-0.17	+0.05	-0.15	±0	-0.16
6	-0.31	-0.15	-0.17	-0.14	-0.24	-0.12
7	-0.39	-0.15	-0.31	-0.14	-0.35	-0.13
8	-0.07	-0.14	-0.23	-0.14	-0.15	-0.12
9	+0.09	-0.10	-0.06	-0.10	+0.01	-0.10
10	-0.03	+0.04	+0.05	-0.07	+0.01	-0.02
11	-0.09	+0.14	+0.06	-0.04	-0.01	+0.04
12	+0.29	+0.20	-0.17	-0.06	+0.05	+0.07
13	+0.43	+0.23	-0.10	-0.08	+0.15	+0.07
14	+0.40	+0.33	-0.15	-0.07	+0.13	+0.12
(New) 15	+0.14	+0.31	-0.05	-0.04	+0.05	+0.13
16	+0.38	+0.29	+0.10	+0.01	+0.23	+0.15
17	+0.21	+0.23	-0.02	+0.07	+0.10	+0.15
18	+0.33	+0.27	+0.18	+0.13	+0.25	+0.20
19	+0.09	+0.26	+0.12	+0.16	+0.11	+0.21
20	+0.34	+0.19	+0.29	+0.25	+0.31	+0.22
21	+0.31	+0.08	+0.21	+0.27	+0.26	+0.18
22	-0.14	-0.01	+0.46	+0.31	+0.17	+0.16
23	-0.21	-0.07	+0.25	+0.25	+0.03	+0.10
24	-0.37	-0.14	+0.36	+0.20	+0.01	+0.04
25	+0.05	-0.11	-0.01	+0.07	+0.02	-0.02
26	-0.02	-0.07	-0.08	+0.01	-0.05	-0.03
27	-0.01	-0.01	-0.18	-0.15	-0.10	-0.07
28	+0.02	-0.06	-0.06	-0.19	-0.02	-0.13
29	-0.10	-0.08	-0.30	-0.22	-0.20	-0.15

first portion should contain an equal period on either side of the middle of June and so the two asymmetries should balance; the portion for August to October should show an asymmetry much more definite than the six months curve.

It will be seen that both these results have been obtained, thus leaving practically no doubt that the asymmetry of the curve is due to the causes previously suggested.

The variance due to the lunar influences has been calculated from the smoothed curves for the period May-July and for the period August-October for all insects. The total variance per lunar month is 0.0996 in the first case and 0.0916 in the second or a mean variance of about 0.0033 per day.

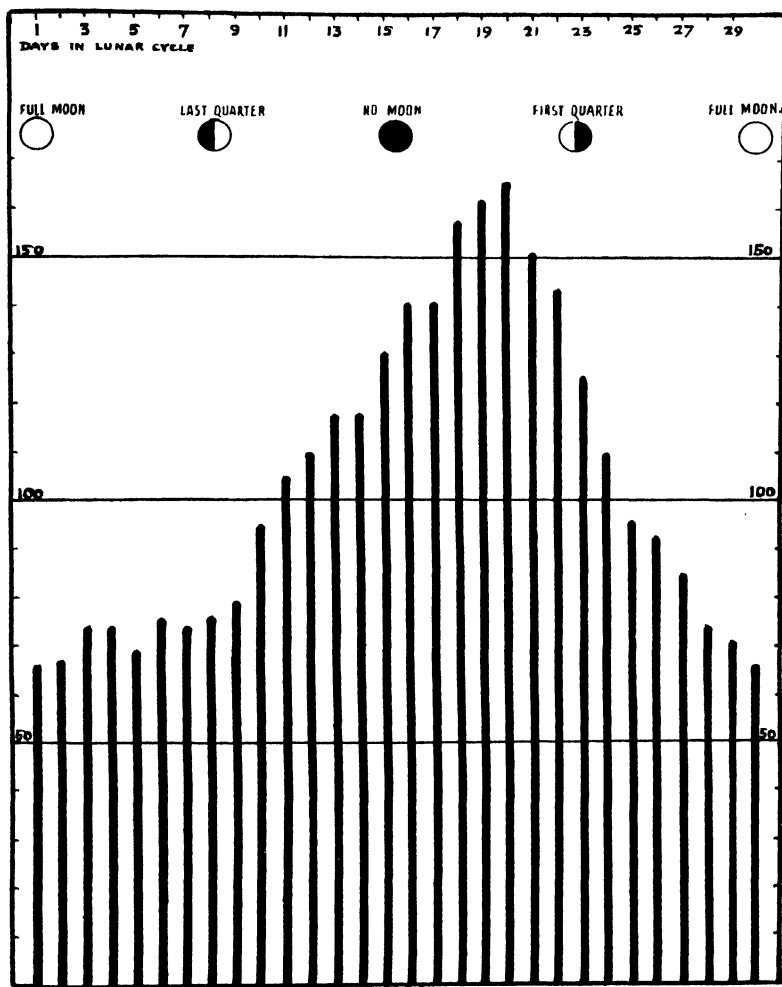
Another method of analysis of the lunar effect, this time also introducing the modifying effect of cloud, consists of dividing the days into nine categories according to the interrelation of two sets of three factors.

(1) According to the moon :—

- (a) nights in week of full moon;
- (b) nights in week of intermediate moon (first and last quarter);
- (c) nights in week of new moon.

(2) According to night cloud :—

- (a) nights with less than 10% cloud (clear);
- (b) nights with 10–90% cloud (intermediate);
- (c) nights with over 90% cloud (cloudy).



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FIG. 17.—Mean number of insects caught on successive days in the lunar cycle in the six summer months (May to October) in the four years. Numbers calculated by re-conversion from mean logarithms.

The mean log. departure for the nights in each of the nine categories is calculated from the catches on the nights in each group, and this figure is converted to a percentage mean catch.

Table 26, A and B, shows the results previously obtained by this method for the NOCTUIDAE and for all insects during 1933–35 (Williams 1936a). Table
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26, C shows the number of nights which occurred in each category in the four summers of 1933-36. There is a total of 703 nights with a minimum of 43 in any one group (the "no-moon; cloudy").

One of the remarkable differences between the results previously obtained for the "NOCTUIDAE" and "all insects" was the very high numbers obtained for the latter in the "no-moon; cloudy" group, or under optimum conditions of flight. The figure for the NOCTUIDAE under these conditions is 184% of the mean, while for "all insects" it is 381%.

TABLE 26.

Percentage catch of all insects under various conditions of moon and night cloud.

	A				B				C			
	NOCTUIDAE, 1933-35				All insects, 1933-35				No. of days, 1933-36			
	Clear	Int.	Cloudy		Clear	Int.	Cloudy		Clear	Int.	Cloudy	
Full moon	42	49	95	54	63	69	115	74	56	77	45	178
Int. moon	92	133	141	120	70	105	129	97	103	164	94	361
No moon	128	144	184	146	81	138	381	151	52	69	43	164
	80	110	140		71	100	166		211	310	182	

	D				E				F			
	All insects, 1933				All insects, 1934				All insects, 1935			
Full moon	145	74	118	107	47	87	83	64	24	56	123	57
Int. moon	68	66	120	78	78	135	120	111	71	115	155	104
No moon	93	107	309	117	87	132	323	144	68	218	468	194
	88	77	144		67	122	147		57	92	203	

	G				H			
	All insects, 1936				All insects, 1933-36			
Full moon	38	48	234	77	57	62	148	76
Int. moon	66	105	151	92	71	105	135	100
No moon	162	148	186	161	93	141	323	151
	71	90	181		71	97	170	

Table 26, D, E, F, and G, shows the values obtained for all insects in each of the four years separately, including 1936 not previously considered. It will be seen that the high activity in "no-moon; cloudy" occurs very definitely in each of the first three years but is not well marked in 1936. The new combined total for the four years (Table 26, H) still shows very considerable activity in this section.

If cloud is neglected the average % catch of all insects over the four years is 76 on nights in the week of full moon, 100 on intermediate nights and 151 on nights of the week of no moon. This difference has been shown to be independent of temperature.

If the phase of the moon is neglected the catch averages 71% on clear nights, 97% on intermediate nights and 170 on cloudy nights. This difference, however, is associated with a temperature difference of about 4° F. between the warmer cloudy and the cooler clear nights.

TABLE 27.

Percentage catch of certain families of insects under various conditions of moon and night cloud.

GEOMETRIDAE				PSYCHODIDAE				CECIDOMYIDAE				CERATOPOGONINAE			
1933				1933				1933				1933			
104	122	118	112	90	137	129	110	141	93	158	129	96	129	100	107
82	92	119	65	49	88	86	72	66	104	89	85	69	93	122	92
115	93	195	119	109	75	179	105	93	82	174	103	152	103	246	144
95	97	132		70	91	109		90	95	116		90	102	138	
1934				1934				1934				1934			
55	78	54	62	55	58	51	55	62	58	49	58	54	124	79	79
111	126	93	115	96	123	91	108	107	138	113	123	95	112	120	110
110	150	306	162	97	135	437	162	116	141	389	170	92	119	398	150
90	119	126		81	108	140		92	116	145		79	116	186	
1933 and 1934				1933 and 1934				1933 and 1934				1933 and 1934			
66	93	76	76	70	84	84	77	92	70	92	84	71	125	89	90
92	113	113	107	68	104	86	86	82	121	95	101	78	104	121	98
117	120	296	153	101	97	287	131	104	104	256	131	116	108	315	147
89	112	141		75	99	120		90	104	125		84	109	154	

Data are also available for several other groups of insects for 1933 and 1934 and this is shown summarised in Table 27 for the families GEOMETRIDAE, PSYCHODIDAE, CECIDOMYIDAE and CERATOPOGONINAE. It will be noted that in all four groups in 1933 the "full moon" figures are rather larger than the intermediate moon figures and in two families were actually larger than the "no moon" figures. This is due to the fact that in 1933 in five out of the six summer lunar periods heat waves occurred about full moon, making this period distinctly warmer than at no moon. Somewhat the reverse happened in 1934 so that the combined totals for 1933 and 1934 eliminated most of this source of error.

It will be seen that higher catches occur in the "no-moon; cloudy" section in all four groups than in the NOCTUIDAE previously dealt with, and also higher catches in the "full moon; clear."

The explanation previously suggested was that the NOCTUIDAE are more generally tolerant of conditions of flight but dislike the worst conditions of full moon and little or no cloud: the other insects are generally somewhat intolerant of unfavourable conditions but develop great activity under the optimum conditions of "no moon and full cloud." This explanation appears to hold for the present results also.

The GEOMETRIDAE are a family of Lepidoptera. The PSYCHODIDAE and CECIDOMYIDAE are families of Diptera. The former particularly came in in very large numbers, and made up about 70,000 of the 73,000 insects caught on the night of the highest catch in the four years. The CERATOPOGONINAE is a sub-family of the family CHIRONOMIDAE and includes a number of small blood-sucking species mostly with mottled wings. On the data available the CERATOPOGONINAE show the highest increase under conditions of "no moon and cloud."

Wind.

Wind records are taken continuously at Rothamsted by means of a Dines Anemograph, which records simultaneously the direction and force of the wind. The vane is at a height of about 70 feet above the ground on one of the main buildings of the Experimental Station.

An examination of a large number of charts shows that the calmest time of the day is during the hours of darkness (when the trap is working) and particularly after midnight.

For purposes of analysis the wind conditions during the night were divided into six artificial groups shown in Table 28.

TABLE 28.

Definition of six wind groups adopted for analysis.

Group	Wind	Approximate Beaufort scale
1	Dead calm, no trace of movement of pen of recording instrument	0
2	Almost calm, slight pen movements indicating velocities seldom over 2 m.p.h.	Just above 1
3	Velocities frequently 2-5 m.p.h.	Distinctly less than 2
4	Velocities frequently 5-10 m.p.h.	Rather less than 3
5	Velocities frequently 10-20 m.p.h.	4
6	Velocities frequently over 20 m.p.h.	5 and over

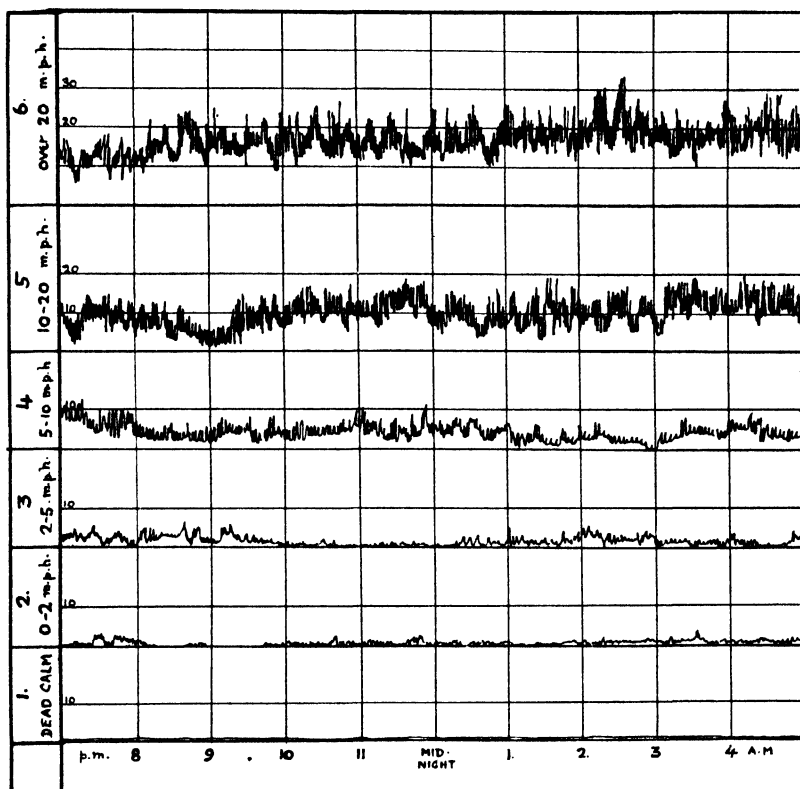
TABLE 29.

Frequency of occurrence of different wind groups in different months of the year, and mean wind value for each month.

Wind group	Frequency of different wind intensities						Mean wind group
	1	2	3	4	5	6	
March (4 years)	39	14	14	29	16	4	2.8
April	28	10	26	26	23	3	3.1
May	30	20	14	15	26	1	2.8
Spring total (338 nights) .	97	44	54	70	65	8	
June	52	14	12	15	12	2	2.3
July	52	23	15	15	15	1	2.2
August	65	17	15	13	9	0	2.0
Summer total (347 nights) .	169	54	42	43	36	3	
September	38	22	13	28	16	3	2.8
October	22	17	10	28	34	12	3.6
November	25	15	23	22	28	6	3.3
Autumn total (362 nights) .	85	54	46	78	78	21	
December	21	19	13	24	40	5	3.5
January	20	18	12	25	33	12	3.5
February	23	12	12	20	32	8	3.5
Winter total (349 nights) .	64	49	37	69	105	25	
Four years total (1396 nights)	415	201	179	260	284	57	3.0

Typical charts from the anemograph for nights in each class are shown in fig. 18. These groups do not correspond exactly to the Beaufort scale, as I was attempting to get a scale which increased its range more or less geometrically in each successive group; the approximate Beaufort number is shown in the last column of the table.

Most of the nights were relatively easy to classify, but a number were difficult and a few impossible. The latter were omitted from the calculations. The chief difficulty was due to rapid change in wind conditions during a single



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FIG. 18.—Typical wind charts from Dines anemograph for nights in the six classes into which the wind conditions have been divided.

night. In making a decision conditions before midnight (when the greatest number of insects are flying) were generally allowed to count more than conditions after midnight, and the wind was rather over- than under-estimated in order that the wind effect would not be exaggerated. In some doubtful cases the night distribution of the insects on that night was also taken into consideration.

Table 29 shows the frequency of occurrence of the nights in each class in each month of the year, together with seasonal and annual summaries. It will be seen that the annual distribution curve has two peaks, one at "calm" and one with winds 10-20 m.p.h. (Group 5). Of the total of 1396 nights, 415

TABLE 30.

Log. catch departure from normal, and minimum temperature departure from normal, in the different wind groups in each month of the year. Based on 4 years data.

Wind group	Catch log. departure						Temperature departure					
	1	2	3	4	5	6	1	2	3	4	5	6
March .	+0.26	+0.06	-0.06	-0.07	-0.33	-0.53	-1.4	-0.3	+0.3	+0.7	+1.3	-0.3
April .	+0.28	+0.29	+0.10	-0.13	-0.24	-0.93	-2.1	-1.6	-1.4	+1.3	+1.1	+7.5
May .	+0.17	+0.08	-0.11	-0.28	-0.13	-1.17	-0.5	-0.2	+1.5	-0.5	+0.6	-3.6
Spring .	+0.24	+0.12	+0.00	-0.14	-0.22	-0.76	-1.3	-0.5	+0.4	+0.7	+1.0	+2.2
June .	+0.36	-0.37	-0.14	-0.10	-0.80	-1.02	+1.2	-2.0	-1.0	-0.1	-0.5	+0.9
July .	+0.24	+0.06	+0.12	-0.38	-0.62	-0.81	-0.9	+0.5	-0.4	+1.5	+1.4	-0.8
August .	+0.15	-0.08	+0.04	-0.31	-0.54	-	-0.4	-1.0	+1.4	-0.2	+1.6	-
Summer .	+0.24	-0.10	+0.02	-0.26	-0.60	-0.96	-0.0	-0.6	+0.0	+0.4	+0.8	+0.3
September .	+0.02	+0.07	+0.10	+0.05	-0.21	-0.75	-1.6	-0.5	+1.0	+1.4	+1.0	+0.2
October .	+0.01	-0.08	+0.36	+0.19	-0.08	-0.40	-1.4	-3.2	+1.4	+0.6	+0.5	+2.5
November .	+0.05	-0.11	+0.21	+0.11	-0.17	-0.52	-2.1	-3.6	-1.4	+2.1	+1.5	+4.2
Autumn .	+0.03	-0.03	+0.22	+0.12	-0.14	-0.48	-1.7	-2.3	-0.0	+1.3	+1.0	+2.7
December .	+0.23	-0.06	-0.01	+0.08	-0.04	-0.52	-2.3	-4.0	-3.3	+1.0	+2.8	-0.6
January .	-0.76	+0.17	+0.28	+0.14	-0.09	-0.40	-5.0	-1.8	+1.5	+0.3	+2.0	+0.9
February .	+0.01	+0.75	-0.11	-0.17	+0.01	-0.26	-1.8	0.9	-0.8	-0.0	+1.2	+0.4
Winter .	+0.06	+0.19	+0.06	+0.03	-0.03	-0.34	-3.0	-1.6	-0.9	+0.5	+2.1	+0.5
Whole year	+0.17	+0.03	+0.07	-0.04	-0.18	-0.48	-1.1	-1.3	-0.1	+0.8	+1.3	+1.5

(30%) were calm and 57 (4%) had winds of over 20 m.p.h. Of the 415 calm nights 254 (61%) occurred in the summer and autumn; while of the 57 stormy nights 46 (80%) occurred in the autumn and winter months.

The method of analysis consisted in placing in the correct wind group the value of the catch (log. departure from 29-day mean) for each of the nights and finding the average catch departure for all nights in that category. The values for each month (4 repetitions in 4 years) have been kept separately.

Table 30 shows the results of this analysis for all insects. Thus from the top line one sees that in all the four months of March the log. catch was 0.26 above normal on calm days, 0.06 above normal with wind group 2 (0-2 m.p.h.), 0.06 below normal with wind 3, 0.07 below normal with wind 4, 0.33 below normal with wind 5 and 0.53 below normal for wind group 6.

The final values for the whole period calculated from nearly 1400 nights are shown in the lowest line of the table, and also graphically in fig. 19, A and B. The general result is that on calm nights the log. catch is 0.17 above normal and on stormy nights 0.48 below or an average change of 0.13 per group. The values of each group expressed as actual numbers with the catch on calm nights as 100 are as follows:—

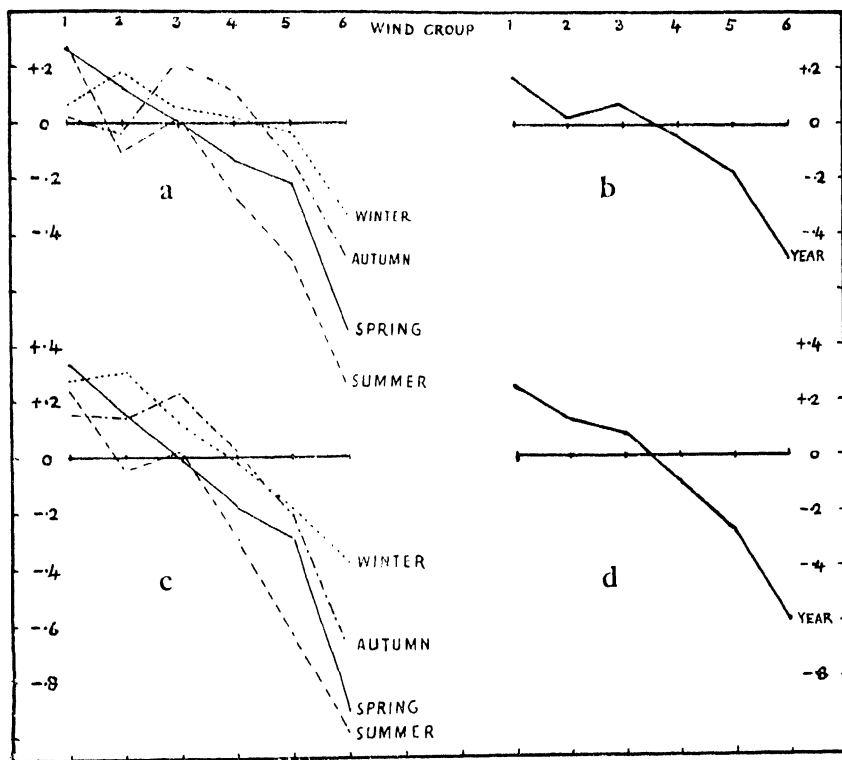
Wind group	.	.	1	2	3	4	5	6
Mean catch	.	.	100	73	81	63	46	22

It will be seen that the sequence is regular except for the fact that catch in wind group 3 is slightly above that in wind group 2. The catch on nights of strong wind is only just above one-fifth of that on calm nights. The quite definite reduction in catch between nights of dead calm and nights with winds only 1-2 m.p.h. is unexpected, particularly as these very light winds are measured at about 70 feet above the ground. It was hardly expected that the difference would be noticed on the flight of insects within a few feet of the ground.

An examination of the meteorological records shows that there is a distinct association between wind force and minimum night temperature, and that, in general, windy nights are warmer than still nights. To study this effect the

mean temperature departures have been calculated for all the groups of nights as used above for catch classification and the results are shown in the second half of Table 30.

From the lower line of the table it will be seen that throughout the whole of the four years calm nights are distinctly cooler than windy nights. The most windy nights are about 1.5° F. above the normal and the calmest nights about 1.1° F. below normal, a total difference of about 2.6° F. This difference is most marked in the autumn, winter and spring months and is much less marked in the summer.



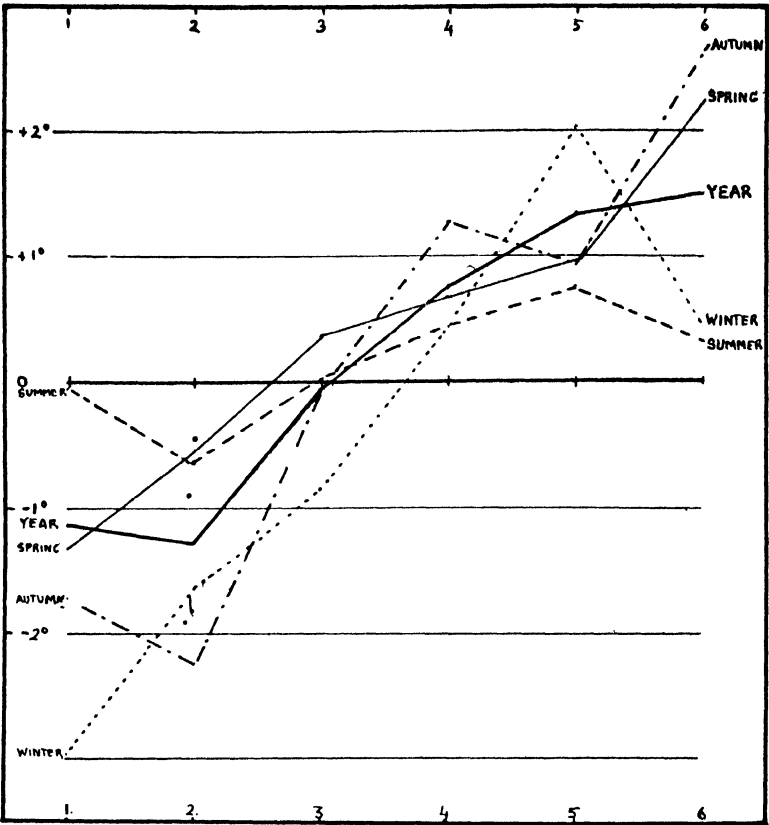
19

FIG. 19.—Departures from normal of the log. catch on nights of different wind intensity during the four years: (a) different seasons uncorrected for temperature; (b) all four years uncorrected for temperature; (c) different seasons corrected for temperature; (d) all four years corrected for temperature.

It will also be seen that on an average the nights with very slight winds (Group 2: 0-2 m.p.h.) were slightly colder than the dead calm nights and distinctly colder than the nights with stronger wind (2-5 m.p.h.).

The relation of wind to temperature is shown graphically in fig. 20.

Since it is known that minimum night temperature is affecting the catch, it follows that temperature and wind are normally working against one another, and that but for temperature the true wind effects would be greater than those shown. An approximate correction can be made by allowing a regression in



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Fig. 20.—Departures from normal of the minimum temperature on nights of different wind intensities during the four years.

TABLE 31.

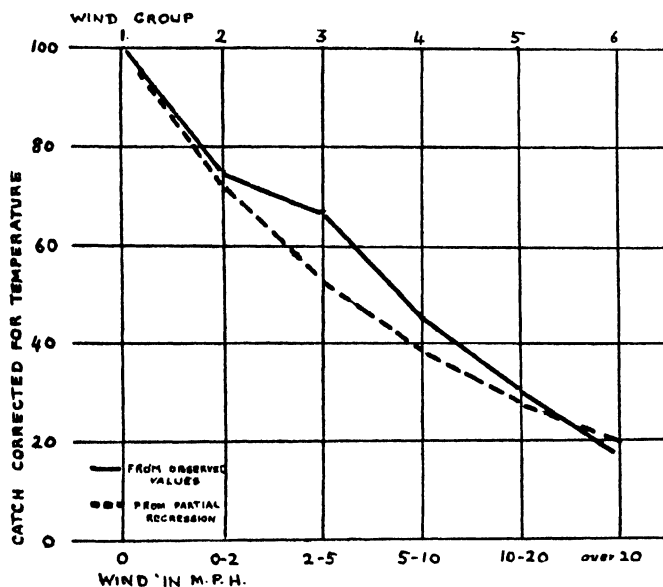
Mean departure of catch, corrected for temperature, in each wind group in the different seasons.

Wind group	1	2	3	4	5	6
Spring	+0.33	+0.16	−0.03	−0.19	−0.29	−0.92
Summer	+0.24	−0.05	+0.02	−0.29	−0.66	−0.98
Autumn	+0.16	+0.14	+0.22	+0.02	−0.21	−0.68
Winter	+0.28	+0.31	+0.12	−0.01	−0.18	−0.38
Whole year { Log. dept. . . .	+0.25	+0.13	+0.08	−0.09	−0.28	−0.60
{ Number	100	75	67	45	30	18
Calculated from { Log. dept. . . .	+0.28	+0.14	0	−0.14	−0.28	−0.42
partial regression { Number	100	73	53	39	28	20

temperature of -0.75 per $^{\circ}\text{F.}$, which has already been found to be near the truth. If this is done the final results are as shown in Table 31 and fig. 19, C and D.

An interesting point is that the bend in the uncorrected curve, due to Group 3 having a slightly higher mean catch than Group 2, is now eliminated and the new curve corrected for temperature is almost regular.

Fig. 21 (solid line) shows the actual numbers caught under different wind conditions (after correcting approximately for temperature) considering the catch at group 1 (dead calm) as 100.



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FIG. 21.—Catch of all insects on nights with winds of different intensities during the four years, expressed as percentages of the catch on dead-calm nights. Solid line giving results from mean catch on different nights with approximate correction for minimum temperature. Dotted line giving results from partial regressions on wind and minimum temperature.

As a check on the above, partial regressions of minimum temperature and wind group have been calculated from the above figures and give :—

Partial regression of log. catch on temperature . . . 0.074 per $^{\circ}\text{F.}$
 " " " " wind . . . -0.138 per wind group.

The catch for each wind group calculated from the above regression is shown in the bottom rows of Table 31 and as a dotted line in fig. 21. The resemblance of the two series calculated by different methods from the available data is sufficiently close to show that the result is somewhere near the truth.

Calculations were also made of the regression of log. catch on wind by the method of difference between successive days. These are summarised in Table 32. They are of course uncorrected for associated temperature effects. The final results for the four years give a negative regression of -0.0946 ± 0.0113

TABLE 32.

Correlations and regressions of log. catch all insects and wind, by method of differences between successive days.

	Cor.	Reg.		Cor.	Reg.
Four March .	-0.36	-0.1517	Spring (11 months).	-0.29	-0.1226
April .	-0.29	-0.1158	Summer (11 months)	-0.28	-0.0946
May .	-0.22	-0.0920	Autumn . . .	-0.09	-0.0391
June .	-0.29	-0.0937	Winter . . .	-0.14	-0.0671
July .	-0.40	-0.1589			
Aug. .	-0.13	-0.0438	1933-34 . .	-0.14	-0.0559
Sept. .	+0.02	+0.0046	1934-35 . .	-0.27	-0.1045
Oct. .	-0.03	-0.0130	1935-36 . .	-0.07	-0.0364 ± 0.0227
Nov. .	-0.20	-0.1042	1936-37 (10 months)	-0.24	-0.1200
Dec. .	-0.27	-0.1472			
Jan. .	-0.17	-0.0769	All four years (46 months) . .	-0.18	-0.0946 ± 0.0113
Feb. .	+0.01	+0.0054			

per single group change, which is slightly lower than the result obtained from the departures from a 29-day mean.

(It will be seen later, p. 294, that when this regression is corrected for both maximum and minimum temperature it becomes -0.144.)

For the month of July in all four years values were calculated for the mean catch of moths of the family NOCTUIDÆ for each of the wind groups. The results were as follows :—

Wind group	1	2	3	4	5	(6)
Log. catch departure						
NOCTUIDÆ . . .	+0.096	+0.013	+0.119	-0.155	-0.191	(-0.180)
Ditto for all insects . .	+0.236	+0.058	+0.123	-0.379	-0.623	-0.840

The value for wind group 6 is from a single night and so is subject to considerable error.

It will be seen that the change in log. NOCTUIDÆ (from +0.119 to -0.191) is very much smaller than that for all insects (from +0.236 to -0.623, neglecting group 6), otherwise that the NOCTUIDÆ are much less affected by wind than the averages of all the other insects flying at the same time. The latter includes a very large number of small Diptera so that the result is in accordance with expectation.

Barometric pressure.

For the purpose of a preliminary analysis of the effect of barometric pressure on the catch, the nights in the six summer months (May to October inclusive) in each of the four years were divided into 9 categories according to whether the barometer was, on the one hand, rising, steady or falling; and on the other hand, high, medium or low. Medium barometer was taken to be between 29.9 and 30.2 inches. The barometer reading was that at midnight, taken from the chart of a recording barograph.

Table 33 shows the number of nights in each of these categories for each year and for the total of the four years, which included 703 nights on which data were available for both catch and barometer. The smallest total number of nights in any one category is 41 in the "low steady" class.

The departures of the log. catch for all insects from a running 29-day mean was entered for each night in its proper category, and the average departure for each category so obtained for each year and for all four years combined.

Table 34, A shows the results for the four years. It will be seen that the

TABLE 33.

Frequency of occurrence of different barometrical conditions. Top line in each successive year 1933-36; bottom line in all four years.

	Rising	Steady	Falling	
High	14, 18, 17, 7 56	20, 25, 23, 25 93	18, 9, 11, 10 48	52, 42, 51, 42 187
Medium	30, 25, 22, 23 100	28, 29, 28, 28 113	33, 31, 33, 23 120	91, 85, 83, 74 333
Low	16, 27, 19, 19 81	6, 9, 14, 12 41	14, 19, 17, 11 61	36, 55, 50, 42 183
	60, 60, 58, 49 227	54, 63, 65, 65 247	65, 59, 61, 44 229	179, 182, 184, 158 703

"high rising" barometer gives the highest catch with a log. departure of + 0.21 above the normal, while "low rising" gives the lowest catch with - 0.37 below the normal.

Table 34, B shows the same figures converted into percentages of a normal catch. Above-normal catches are obtained with high barometer if rising, or steady, and with medium barometer if steady or falling. Below-normal catches are obtained with low barometer; with medium barometer if rising; and with high barometer if falling. This latter result is somewhat unexpected but it occurs in each of the four years of which the table shows the combined total.

The lowest average catch is 43% of the normal (with low barometer rising), the highest average catch is 162% of the normal (with high barometer rising). If the direction of movement is neglected the average catches under high, medium and low barometer are 135; 120; and 51 respectively. If the height of the barometer is neglected the average catches with rising, steady and falling barometer are 74; 129; and 115.

TABLE 34.

Departure of log. catch all insects from normal under various barometrical conditions, in all four years.

	A Catch (log. dept.)				C Min. temperature				E Wind			
	Ris.	Stdy.	Fall.		Ris.	Stdy.	Fall.		Ris.	Stdy.	Fall.	
High	+0.21	+0.20	-0.07	+0.13	-1.6	+0.5	+0.5	-0.01	2.1	2.3	2.3	2.2
Medium	-0.10	+0.17	+0.14	+0.08	-2.1	+1.3	+1.6	+0.4	2.3	2.2	2.7	2.4
Low	-0.37	-0.28	-0.19	-0.29	-2.2	-0.6	+1.4	-0.6	3.3	3.0	3.7	3.3
	-0.13	+0.11	+0.06		-2.1	+0.7	+1.3		2.6	2.4	2.9	2.6
	B Catch (%)				D Catch corr. for temp.				F Catch (%) corr. for temp. and wind			
	Ris.	Stdy.	Fall.		Ris.	Stdy.	Fall.		Ris.	Stdy.	Fall.	
High	162	159	85	135	213	111	77	134	177	125	67	114
Medium	79	148	138	120	114	119	101	111	100	101	101	101
Low	43	52	65	51	62	57	49	57	78	65	70	72
	74	129	115		106	114	90		106	101	100	

In order to see to what extent these differences might be explained by other factors known to be associated with special barometrical conditions, the departures from the normal for minimum temperatures and the average wind intensity (in 6 groups as explained on p. 278) was worked out for each of the nine categories and are shown in Table 34, C and 34, E. With this information and a knowledge of the approximate effect of unit change of temperature and wind (see pp. 262 and 283), it is possible to make a correction for the effect of these factors on the catch, either separately or together.

Table 34, D shows the log. departures for the catch corrected for minimum temperature and converted into percentages; and Table 34, F shows the results corrected for both temperature and wind.

It will be noted that after these corrections have been made low barometer is still associated with low catches; medium barometer gives results almost normal; while high barometer gives catches above normal if the barometer is rising or steady, but well below normal if the barometer is falling. The difference between rising and falling has been much reduced except in cases of high barometer.

Neglecting the direction of movement, the catches with high, medium and low barometer are now 114, 101 and 72 (135; 120 and 51 before correction); and neglecting the height of the barometer the catches with rising, steady and falling barometer are 106, 101 and 100 (74; 129; and 115 before correction).

The low catch with high falling barometer still persists after correction for wind and minimum temperature and is difficult to explain.

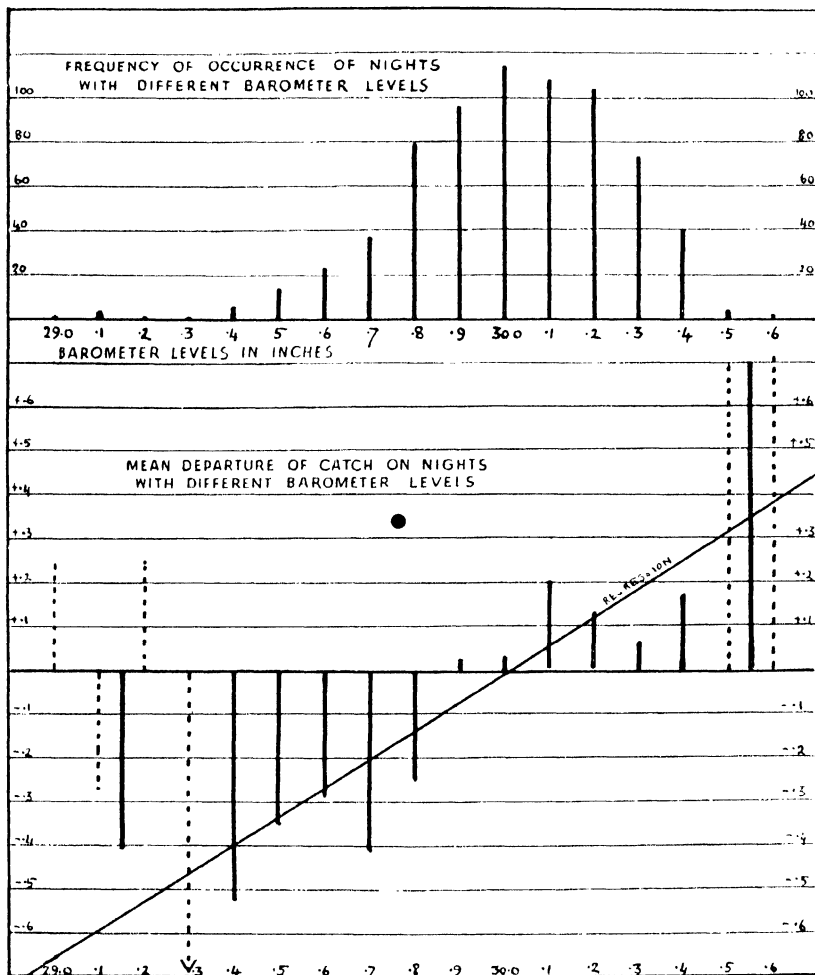
A more complete analysis of the effect of height of barometer, independent of the direction of movement, is shown in Table 35 and fig. 22. On these are shown first the frequency of occurrences of nights with different barometer levels at midnight. It will be seen that the lowest barometer level observed during the four summers was 29.0 inches (recorded once only) and the highest 30.6 inches (recorded twice). The most frequent level is 30 inches, and the curve of distribution is not symmetrical.

TABLE 35.

Frequency of occurrence of different barometrical levels in the summer months of all four years and the mean log. catch departure from normal on these nights.

Barometer level at midnight	Number of occurrences	Mean catch departure
29.0 inches	1	(+0.25)
0.1	4	-0.27
0.2	1	(+0.25)
0.3	1	(-1.73)
0.4	6	-0.52
0.5	14	-0.35
0.6	23	-0.29
0.7	37	-0.41
0.8	79	-0.25
0.9	95	+0.03
30.0	114	+0.03
0.1	108	+0.20
0.2	104	+0.13
0.3	73	+0.06
0.4	40	+0.17
0.5	4	+0.70
0.6	2	(+0.72)

The lower histogram in fig. 22 shows the mean departures of the log. catch from the normal for all the nights with each of these barometer levels. The values obtained from fewer than five readings are shown dotted, with a solid line between those showing the average for all the high and all the low values of this character.



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FIG. 22.—Effect of barometric pressure on catch. Above: frequency of occurrence of nights with different barometer levels in six summer months for four years. Below: mean catch on nights of different barometer levels and regression of catch on pressure.

The regression of the rate of change of catch per unit change of temperature has been calculated from the whole of the available data for the four years and is 0.668 change in log. catch per inch change of barometer. Otherwise a rise of barometer of 0.45 inch is equivalent to adding 0.30 to the log., which is equivalent to doubling the catch.

The regression line is shown across the histogram in fig. 22.

Thunder and lightning.

Thunder and lightning were recorded by the meteorological observers on 40 days during the summer months of the four years. Nearly all cases occurred in the late afternoon or evening. The days were distributed as in Table 36.

TABLE 36.

Frequency of occurrence of thunderstorms in the six summer months of the four years.

	1933	1934	1935	1936	Total
May	1	2	2	1	6
June	4	2	2	4	12
July	2	3	2	4	11
August	0	1	0	1	2
September	1	2	2	1	6
October	0	0	2	1	3
Total	8	10	10	12	40

The mean log. catch of all insects on the nights following these days (averaged from departures from a 29-day mean) was -0.057 , equivalent to a catch of 12% below normal. As the standard error of the mean is ± 0.097 the difference is not significant.

The mean log. catch of all insects on the night previous to these days, *i.e.* the catch during the conditions just preceding thundery weather is $+0.053$ or about 13% above normal. But again the difference is not significant.

If, however, one deals with moths of the family NOCTUIDAE instead of all insects one gets a significant difference as follows.

On the nights following days with thunder the log. catch of NOCTUIDAE (again from departures from a 29-day mean) is $+0.076$ (or 19%) above normal, with a standard error of ± 0.023 .

On the nights preceding thundery weather the log. catch of NOCTUIDAE is $+0.094$ (equivalent to a catch of 24% above normal) with a standard error of ± 0.021 .

That these results are not simply due to temperature is shown by the fact that the average minimum temperature on both days preceding and days following the thundery weather was 0.5° F. below normal.

The figures therefore lend a certain amount of support to the idea widespread among collectors of Lepidoptera that moths may be captured more abundantly during conditions of thundery weather.

ANALYSIS OF SEVERAL FACTORS SIMULTANEOUSLY.

Interrelation of climatic factors.

Owing to the fact that the different climatic factors are themselves correlated, as previously outlined on p. 242, each factor does not appear to produce the same effect when it is considered singly, neglecting the others, as when it is considered together with other factors and the effect of each is assessed independently. Thus I have found that a rise of one degree in minimum temperature (neglecting maximum) is associated with an increase in log. catch of 0.060, while an increase of one degree in maximum temperature (neglecting minimum) is associated with an increase in log. catch of 0.042. But it is known

that, in general, warm days are followed by warm nights—or, in other words, that there is a positive correlation between the two—so that some of the effect found on warm nights must be due to the warmth of the previous day, and some of the effect credited to the maximum temperature must be due to the following minimum. Thus when an analysis is made that assesses independently the effect of two positively correlated factors (such as maximum and minimum) the effect of each is slightly less than when they are considered independently. If, on the other hand, they are negatively correlated (or if the correlation of one with the trap is positive and the other negative) then the new values will be larger than before. The more closely the climatic factors are correlated the more difficult it is to assess to each their proper value. The mathematical process necessary is that of “partial regressions” and will be found described in any statistical textbook.

For an understanding of the results, however, it is necessary to realise the extent to which the climatic factors are themselves correlated.

Values for the correlation of the four factors, maximum and minimum temperature, wind and 9 p.m. relative humidity, are shown in Table 37, based on the results of all four years.

TABLE 37.

Correlation of temperature, wind force and relative humidity, based on difference of successive days over four years.

	Max. T.	Wind	R.H. 9 p.m.
Minimum temperature	+0.32	+0.32	+0.04
Maximum temperature	—	+0.18	—0.18
Wind	—	—	—0.08

It will be seen that the correlations are positive between maximum and minimum temperature and wind; negative between 9 p.m. R.H. and maximum temperature (in other words, the hotter the previous day the dryer the evening); negative but of doubtful significance between wind and 9 p.m. relative humidity; and doubtfully positive between minimum temperature and 9 p.m. relative humidity.

The correlation between the minimum temperature on one night and that of the previous night on 12 summer months (351 days) is 0.36, while with the night before that (2 nights previous) the correlation is only 0.13.

Other correlations have already been mentioned but have not been calculated as a mathematical expression. Thus cloudy nights have higher minimum temperature: the minimum temperature is slightly higher with a falling barometer than with a rising one (probably owing to the effect of cloud); and the wind is greater with a low barometer than with a high barometer.

It is these numerous interrelations that make the analysis of the partial regressions which follows so difficult and which gives the results relatively high errors in spite of the large number of observations on which they are based.

Temperature, moon and cloud.

An examination has been made to see if the regression of log. total insects on minimum temperature is different under different conditions of cloud and moonlight. Unlike all the others which follow, this was not done by partial regressions but by dividing the nights up into the nine moon-cloud categories

as already explained (p. 274) and then calculating the regressions on temperature for each group, and for combinations of groups.

The results for the three years 1933-35 are shown in Table 38. In each square the upper figure is the total number of days in that category; the number on the left is the correlation; and the number on the right the regression. It will be seen that there are no large differences between the groups, except that all cloudy nights give a regression of 0.023 while all intermediate and all clear nights give a regression of 0.051 and 0.053. When, however, the errors are calculated, it is found that the difference between cloudy and intermediate is 0.028 and the error of the differences 0.0175. The difference is therefore 1.6 times the standard error and not significant, as once in ten times this difference could be obtained by chance.

There is therefore, from these data, no proof of a difference of regression under different conditions of cloud and moon.

TABLE 38.

Correlations and regressions of log. catch on minimum temperature in the three years 1933-35, in different cloud and moon conditions.

	Clear		Intermediate		Cloudy	
	Cor.	Reg.	Cor.	Reg.	Cor.	Reg.
Full moon	45 0.47	0.057	43 0.34	0.059	30 0.14	0.023
					0.39	0.057 \pm 0.013
First and last quarter	82 0.42	0.049	107 0.36	0.050	56 0.13	0.020
					0.36	0.047 \pm 0.008
New moon	41 0.61	0.060	46 0.26	0.035	37 0.21	0.032
					0.42	0.049 \pm 0.010
	168 0.47 \pm 0.010		196 0.34 \pm 0.010		123 0.15 \pm 0.014	

Minimum temperature on previous nights.

Calculations have been made to show to what extent the catch is affected by the minimum temperatures of the nights previous to the one on which the catch is made.

Table 39 shows the partial regressions of log. total catch of all insects on the minimum temperature of the current night, the previous night and the night before that. Also, for comparison, the regression on the current night only.

It will be seen that on the total twelve summer months the effect per degree for the current night is 0.0662 and for the previous night 0.0150, while the regression on the second night previous is very small indeed and not significant.

Thus if the two nights only are considered, about four-fifths of the effect is due to the temperature on the current night and about one-fifth to that on the previous night.

An analysis of variance shows that the mean trap variance is 0.387 per day, of which 0.282 is residual error when allowance has been made for the three regressions. Approximately 27% of the total variance is accounted for by these regressions. When, however, the residual variance is calculated from the

simple regression on minimum temperature for the current night only the residual variance is almost exactly the same—0.284.

This shows that little or no gain in accuracy in prediction is obtained by using the extra information from the previous night.

TABLE 39.

Regressions and partial regressions, all insects (log. departure from monthly means) and minimum temperature, in three summer months of four years.

	Regression on current night only	Partial regressions on		
		Current night	Previous night	2nd night previous
May 1933	0.1107	0.1004	0.0322	0.03006
1934	0.107	0.0952	0.0295	—0.01030
1935	0.106	0.1113	—0.0111	0.00352
1936	0.0965	0.0898	0.0067	0.03270
All 4 Mays	0.1052	0.0981	0.0089	+0.0097
All 4 Julys	0.0598	0.0579	0.0118	—0.01649
All 4 Septembers	0.0561	0.0504	0.0170	—0.00047
12 months as above	0.0716	0.0662	0.0150	+0.00041

Temperature at 9 p.m. and different methods of expressing humidity.

For the months of June and July 1933 calculations were made of the partial regressions for the total catch on 9 p.m. temperature and the 9 p.m. humidity. The latter was expressed in three different ways: (1) Relative Humidity as percentage, (2) Saturation Deficiency (in inches), and (3) Absolute Humidity as Vapour Pressure in inches (see p. 241).

It will be seen from Table 40 that the regression on temperature is lowest in both months when vapour pressure is the second factor, and highest when saturation deficiency is the second factor. Further, in June the mean variance is 0.2782 and the residual variance is lowest with saturation deficiency and highest with vapour pressure. In July the residual variance is lowest with relative humidity and highest with vapour pressure.

In June 1933 a further calculation was made, using the insects in the first period of the night only as the dependent variable. These were the insects caught round about 9 p.m., when the temperature and humidity observations were taken. This again shows the highest temperature effect with saturation deficiency and the lowest with vapour pressure; on the other hand, the analysis of variance shows the lowest residual variance when using vapour pressure.

The figures indicate that for the purpose of this type of analysis, there is very little difference in accuracy of result to be obtained by using any one method in preference to another.

This being the case, calculations were completed for partial regressions of total catch on 9 p.m. temperature and 9 p.m. relative humidity for the fifteen months, May 1933–July 1934. (After the latter month the 9 p.m. observations were unfortunately discontinued.) The results are shown in Table 41. For the whole year, the partial regression on minimum temperatures was 0.0834 per degree and on relative humidity 0.0164 per 1% change. Thus 1° F. has the same effect as 5.6% change in relative humidity. Also a rise of either 3.4° F. or 18% in relative humidity will double the catch.

TABLE 40.

Comparison of partial regression of catch on temperature and on different methods of expressing the humidity of the atmosphere, all from departures from monthly means.

1933	June		July	
	Regression on		Regression on	
	Temp.	Moisture	Temp.	Moisture
Total catch :				
Mean variance . . .		0.2782		0.4327
9 p.m. Temp. and rel. humidity . . .	0.124	0.0327	0.155	0.0283
Residual variance . . .		0.0860		0.1739
9 p.m. Temp. and sat. deficiency . . .	0.159	—0.300	0.177	—0.186
Residual variance . . .		0.0766		0.1750
9 p.m. Temp. and vapour pressure . . .	0.041	0.0650	0.086	0.0394
Residual variance . . .		0.1068		0.1843
Catch in 1st period only :				
Mean variance . . .		0.3502		
9 p.m. Temp. and rel. humidity . . .	0.1005	0.0215		
Residual variance . . .		0.2233		
9 p.m. Temp. and sat. deficiency . . .	0.1234	—0.1923		
Residual variance . . .		0.2240		
9 p.m. Temp. and vapour pressure . . .	0.0411	0.0560		
Residual variance . . .		0.2132		

The mean variance of the catch for this year was 0.4139 and the residual variance after subtracting the effects of 9 p.m. temperature and 9 p.m. relative humidity was 0.2616, showing that about 37% is accounted for. This is higher than the figure 24% obtained by using maximum and minimum temperature (see p. 293), but the latter was on four years analysis while this is only on one year.

TABLE 41.

Partial regressions of log. total catch on 9 p.m. temperatures and relative humidity (from departures from monthly means).

	Temp.	Rel. H.		Temp.	Rel. H.
1933 May . . .	0.1307	0.0048	1934 May . . .	0.0762	0.0035
June . . .	0.1235	0.0327	June . . .	0.0554	0.0025
July . . .	0.1545	0.0283	July . . .	0.0810	0.0037
Aug. . .	0.0700	—0.0001			
Sept. . .	0.0594	0.0165	Six summer months, May to Oct. 1933 . . .	0.0857	0.0150
Oct. . .	0.0505	0.0741	Six winter months, Nov. 1933–April 1934 . . .	0.0792	0.0186
Nov. . .	0.0830	0.0422	Year, May 1933 to April 1934 . . .	0.0834	0.0164
Dec. . .	0.1450	0.0301			
1934 Jan. . .	0.0564	0.0246			
Feb. . .	0.0531	0.0079			
March . . .	0.0907	0.0132			
April . . .	0.0869	0.0097			
			Mean variance for whole year = 0.4139		
			Residual variance = 0.2616		

Maximum and minimum temperatures.

The individual effect of maximum and minimum temperature, acting simultaneously, on the total catch of all insects is shown by the partial regressions in Table 42.

It will be seen that the average effect over the four years is 0.0539 per degree minimum temperature and 0.0215 per degree maximum. These are both smaller than the single regressions but this is to be expected as the maximum and minimum temperatures are themselves correlated and their effects are both positive.

TABLE 42.

Partial regressions of log. catch on maximum and minimum temperatures (from differences between successive days) with simple regressions for comparison.

All four years	Partial regressions		Simple regressions for comparison	
	Minimum	Maximum	Minimum	Maximum
March . . .	0.0704	-0.0095	0.069	0.005
April . . .	0.0302 \pm 0.0129	0.0305 \pm 0.0131	0.042	0.043
May (3) . . .	0.0664	0.0179	0.074	0.038
June (3) . . .	0.0700 \pm 0.0136	0.0278 \pm 0.0108	0.080	0.044
July . . .	0.0460	0.0342	0.050	0.039
August . . .	0.0539 \pm 0.0107	0.0275 \pm 0.0105	0.063	0.045
September . . .	0.0405	0.0272	0.045	0.043
October . . .	0.0627 \pm 0.0115	0.0109 \pm 0.0200	0.066	0.063
November . . .	0.0556	0.0022	0.056	0.022
December . . .	0.0400 \pm 0.0232	0.0673 \pm 0.0245	0.060	0.091
January . . .	0.0511	0.0267	0.063	0.055
February . . .	0.0819 \pm 0.0170	0.0089 \pm 0.0167	0.079	0.017
All 4 years . . .	0.0539 \pm 0.0038	0.0215 \pm 0.0044	0.060	0.042

Analysis of variance shows that the total mean variance of the trap is 0.5885 per day, of which 0.1390 (24%) is accounted for by the two partial regressions. With the single regression 0.0964 (16%) is accounted for by minimum temperature only, and 0.0350 (6%) by maximum temperature only.

Calculations have also been made of the partial regressions on maximum and minimum temperature for the 4 summer months (June to September) for the catches of Lepidoptera only. They are shown in Table 43. It will be seen that the values per degree are smaller with the Lepidoptera only than

TABLE 43.

Partial regressions of Lepidoptera only on maximum and minimum temperature, in the four summer months of all four years, with figures for all insects for comparison.

Lepidoptera only	Partial regressions	
	Minimum	Maximum
June (3)	0.063 \pm 0.011	0.031 \pm 0.009
July (4)	0.041	0.030
August (4)	0.042	0.019
September (4)	0.026	0.014
Four summers (15 months)	0.0373 \pm 0.0039	0.0251 \pm 0.0041
Four summers. All insects for comparison	0.0484 \pm 0.0052	0.0304 \pm 0.0056

with the total catch all insects, but that the difference in the value for minimum temperature is barely significant and the difference for the maximum temperature is quite definitely not significant.

Simultaneous effect of maximum and minimum temperature and wind.

Table 44 shows the partial regressions on maximum and minimum temperature and wind, calculated by the method of differences between successive days over the four years.

It will be seen that there is little difference between the regressions on minimum temperature in the four seasons; with the maximum temperature spring and autumn are low and summer and winter higher, but the differences are doubtfully significant; with the wind, spring gives a higher value and autumn low, but again the differences are only just on the verge of significance and might be due to accident. More results must be obtained before these points can be settled.

For the whole period of the four years the regression on minimum temperature is 0.0696 ± 0.0038 ; on maximum is 0.0225 ± 0.0042 and on wind -0.1439 ± 0.0103 . Thus the catch is doubled by an increase of minimum temperature of about 4.4° F.; by an increase in maximum temperature of about 13.4° F.; and by a change of wind of approximately two groups. Each of these is of course considered as acting quite independently of the others and free from the normal correlation that exists between them.

Analysis of variance shows that the mean trap variance over the four years is 0.5775, and after allowing for the three regressions the residual variance is 0.4170 or 72%. Thus 28% of the variance is accounted for by these three factors over the four years.

TABLE 44.

Partial regressions of log. catch on maximum and minimum temperature and wind for the four years 1933-37, from differences between successive days.

	Minimum temp.	Maximum temp.	Wind
Spring (March-May) .	0.0739	0.0128	-0.1883
Summer (June-Aug.) .	0.0654	0.0307	-0.1423
Autumn (Sept.-Nov.) .	0.0680	0.0185	-0.1283
Winter (Dec.-Feb.) .	0.0709 \pm 0.0093	0.0262 \pm 0.0105	-0.1344 \pm 0.0233
All 4 years .	0.0696 \pm 0.0038	0.0225 \pm 0.0042	-0.1439 \pm 0.0103

Simultaneous effect of four factors : maximum and minimum temperature, wind and 9 p.m. relative humidity.

For the year 1933-34 data were available to calculate the simultaneous effect of four factors which are shown in Table 45. That on minimum temperature is 0.0652 ± 0.0075 ; on maximum 0.0164 ± 0.0077 ; on wind 0.1209 ± 0.0203 , and on relative humidity 0.0063 ± 0.0033 . The regression on humidity is just significantly positive. It is small because of the small unit taken, which is 1% change in relative humidity.

The catch is therefore doubled by 4.6° F. rise in minimum temperature; by 18° F. rise in maximum temperature; by 2.5 fall in wind group and by 47% change in relative humidity.

Analysis of variance shows that out of a mean variance of 0.4921, the

residual is 0.3742 or 76%. Thus about 24% of the variance is accounted for in this year by the four factors.

If relative humidity is neglected and only the other three factors are used for this year the values of the regressions are shown in the last column of Table 44. In this case the residual variance is 0.3781, which is practically indistinguishable from the result with four factors. Thus little or no increase in accuracy of estimation is obtained by adding relative humidity to the list of factors.

TABLE 45.

Partial regressions of log. catch on four weather factors, for year March 1933 to February 1934, from differences between successive days.

	Regression	Three factors only
Minimum temperature . . .	+0.0652 \pm 0.0075	0.0670
Maximum temperature . . .	+0.0164 \pm 0.0077	0.0133
Wind . . .	-0.1209 \pm 0.0203	0.1250
Rel. humidity at 9 p.m. . .	+0.0063 \pm 0.0033	—

Analysis of variance.

I have shown that the effect of different weather conditions on the catch of insects in the trap can be analysed; first for single factors, then for two simultaneously, and finally for three and four factors acting together.

Any factors which can be expressed numerically can be added to the analysis but as each new variable is included for the same trap data, the labour of calculation and the error of assessing the values both become greater. This is particularly so when, as is usually the case, the climatic factors are themselves highly correlated.

I start with the variableness or "variance" of the trap catch, as much as possible of which it is desired to explain.

Using the catch expressed as log. $(n + 1)$ and the method of differences between successive days the total variance (sum of square of differences) in the four years is 775.5919. This is on 1343 days and the mean variance is therefore 0.5775. Otherwise the mean change of the catch from one day to the next throughout the four years was 0.76 (the square root of the mean variance).

This variance is caused by :—

- (1) changes in activity due to weather;
- (2) error of estimation due to using a single trap;
- (3) lunar effect;
- (4) population changes not eliminated by using differences between successive days;
- (5) other causes which must be grouped together as "error."

(1) Using the triple regressions on minimum temperature, maximum temperature and wind (p. 294) I can account for a variability during the four years of 217.7206.

(2) It is shown on p. 234 that the error per night by using a single trap was, on the method of differences, 0.2619 with a variance of 0.0686. This for the 1343 nights accounts for a variance of 92.1298. It is extremely likely that this is an under-estimation as only two months data (September and October 1933) were available from which it could be calculated. During this

period catches were relatively high. I should expect greater differences between the catches of the two traps in the winter, when catches are low.

(3) The lunar effect is very regular and has been shown (p. 272) to vary for all insects between the extremes of -0.18 and $+22$, or a change of 0.40 in a half lunar period of about 14 days. The change, however, is probably more extreme in autumn and spring than in summer and winter. It has already been shown to average approximately 0.0033 per day. This would on the 1343 days only account for 4.4319 of the total variance.

(4) Population changes have been eliminated as far as possible by using differences between successive days, but even this method will not allow for sudden emergence of large numbers of adults for the chrysalis, or for sudden destruction of large numbers by frost, rain or other causes. There is therefore a residual error from this cause which it does not seem possible at present to assess.

The balance sheet, or analysis of variance as it is termed in statistics, is as follows:—

	Degrees of freedom	Total variance	Mean variance
All sources	1341	775.5919	0.5775
Temperature and wind	3	217.7206	
Trap error	1	92.1298	
Moon	1	4.4319	
Residual	1336	461.3096	0.3453 = 60% of original

Thus 60% of the variance still remains unexplained as "error."

Table 46 and fig. 23 show how the mean variance changes from month to month for both trap, temperature and wind. It will be seen from this that the variance of the trap makes a very sudden rise in October and November and remains

TABLE 46.

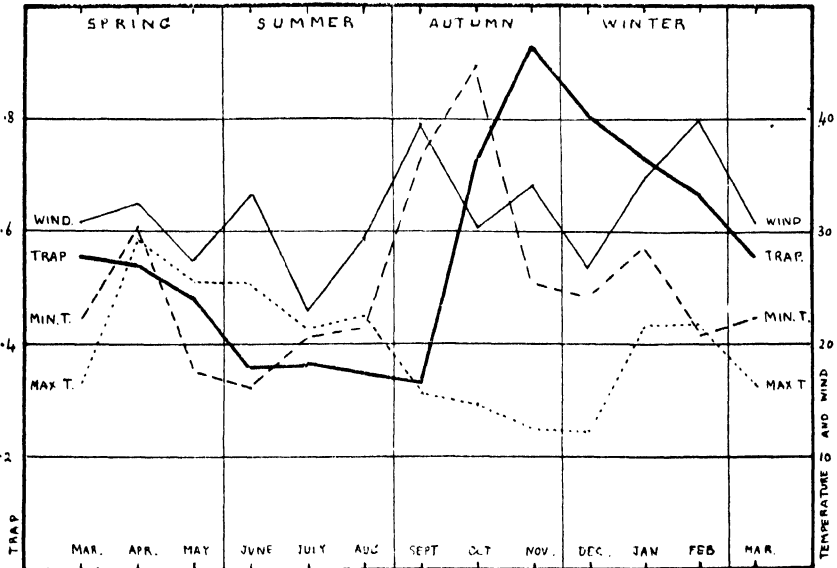
Mean variance per day of trap, maximum and minimum temperature and wind, from differences between successive days.

Trap	Min. T.	Max. T.	Wind	Trap	Min. T.	Max. T.	Wind
March . . 0.5570	22.05	16.55	3.09	Spring . . 0.5283	23.92	23.84	3.05
April . . 0.5358	30.50	29.46	3.26	Summer . . 0.3591	19.61	22.82	2.81
May . . 0.4834	17.81	25.66	2.73	Autumn . . 0.6655	35.83	14.11	3.47
June . . 0.3599	16.08	25.32	3.33	Winter . . 0.7372	24.76	18.37	3.35
July . . 0.3689	20.54	21.43	2.30				
Aug. . . 0.3485	21.36	22.31	2.92	1933-34 . . 0.4921	24.27	20.33	3.07
Sept. . . 0.3339	36.94	15.61	3.97	1934-35 . . 0.4926	25.56	21.56	3.29
Oct. . . 0.7315	44.80	14.71	3.02	1935-36 . . 0.6190	28.50	17.54	3.37
Nov. . . 0.9345	25.43	12.27	3.43	1936-37 . . 0.7353	25.59	18.75	2.94
Dec. . . 0.8038	24.04	12.01	2.68				
Jan. . . 0.7317	28.69	21.70	3.48	All 4 years . 0.5775	26.21	19.58	3.18
Feb. . . 0.6660	20.97	21.88	4.00				

at a high level during the winter. The mean variance for the four Novembers is 0.9345 , which is nearly three times the value for September, when it is lowest. It is during the months of October, November and December that very great numbers of TRICHOCERIDAE (winter-gnats) come into the trap. In fact the mean catch of the trap for November (average of four years) is actually above that of October, in spite of the fall of temperature. Undoubtedly this introduces new factors into the problem the effect of which is at present difficult to estimate.

One of them is undoubtedly a much higher humidity effect, as the *Trichocera* are in very great abundance on damp nights. The effect of this is shown in Table 22 (p. 268) in the much higher regression of insects on humidity in the autumn than at any other period.

If, therefore, one considers that some abnormal factor makes it incorrect to apply the same variance and the same regression to all times of the year,



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FIG. 23.—Annual sequence of variability in the trap catch and in some weather factors; all calculated from difference between successive days.

one may compare with the whole year's analysis of variance just given that for the three summer months from June to August only as follows:—

	Degrees of freedom	Total variance	Mean variance
All sources	326	117.0773	0.3591
Temperature and wind	3	46.9918	
Trap	1	22.3636	
Moon	1	1.0758	
Residual	321	46.6461	0.1453 = 40%

In this case the residual error is only 40%, so that 60% is explained on the known sources of variability.

ESTIMATION AND FORECASTING OF THE POPULATION.

Estimation.

In the previous portion of this paper I have shown that the catch is dependent on both activity and population, and an attempt was made to estimate the effect of activity by eliminating as far as possible the effects of population. The object of the present section is to see if it is possible to perform the reverse operation which is to eliminate the activity effects and so estimate the population changes.

I have shown that the effect of maximum and minimum temperatures, of wind and of relative humidity can be expressed by certain regression values giving the effect on the dependent variable (the catch) of unit change in the independent variable. As these values have been calculated from differences between successive days, they are believed to consist very largely of the activity effect, and to exclude as far as possible longer trends of population change.

The mean log. catch for the month of March in all four years was 0.88, while the mean catches in March in each of the four successive years were 0.97; 0.56; 1.13 and 0.87 respectively. Thus the mean catches departed from their own average by + 0.09; - 0.32; + 0.25 and - 0.01 respectively, as shown in the first column of Table 47.

Part of these differences must be due to the different activity in March of each year, owing to weather conditions; and part to the fact that the population was not always the same. If one considers that other errors are as likely to be positive as negative, the total departure for each month must be made up of the change in activity due to the current weather conditions plus the change in population due, as will be discussed later, largely to previous weather conditions.

But since both the effect of unit change in each of the principal weather conditions on activity, and the extent to which the weather conditions varied from the mean in each month are known, it should be possible to correct for these departures in activity and be left with a value for the catch which would have been obtained under standard conditions. Changes in this should be a measure of population change.

If reference be made to Table 2 (p. 231) it will be seen that in March 1933 the minimum temperature was 0.1° F. below the normal for the four years and the maximum temperature was 3.3° F. above normal. The wind value was 0.27 below normal. From Table 44 will be found the partial regression values for each of these factors. From these two sets of figures it will be seen that the expected departure from normal of the catch in March 1933, due to activity factors is:—

$$\begin{array}{rcl}
 0.1 \times 0.070 & = & + 0.007 \text{ for the minimum temperature} \\
 3.3 \times 0.0225 & = & + 0.076 \text{ for the maximum temperature} \\
 - 0.27 \times - 0.144 & = & + 0.039 \text{ for the wind force} \\
 \hline
 \text{Total} & = & + 0.122
 \end{array}$$

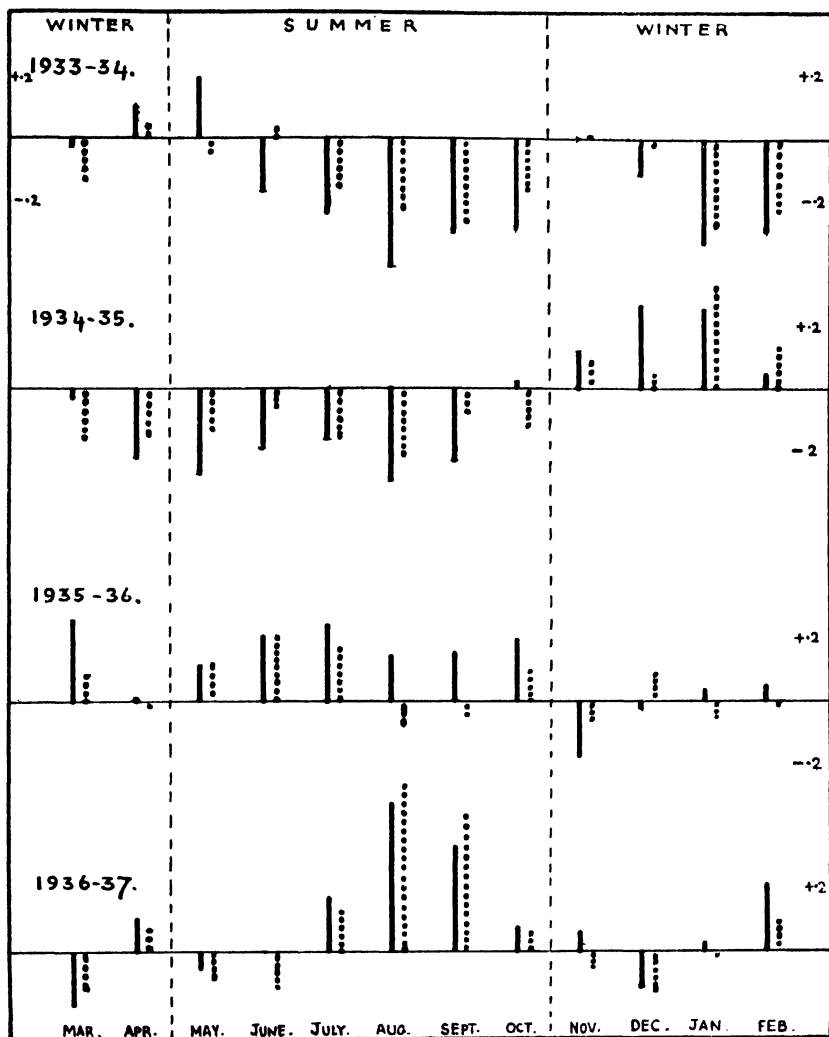
Otherwise that owing to the weather conditions during March the catch would be expected to be 0.12 above normal if the population were normal. Actually the catch was 0.09 above normal or - 0.03 below the expected value. If this reasoning be correct, and no major factor is left out, this - 0.03 should be a measure of the population change from normality for March 1933. Similar calculations give - 0.03, + 0.26 and - 0.17 for March in 1934, 1935 and 1936.

In Table 47 is shown first of all the observed departure from the mean in each of the 48 months; next the expected departure owing to the effect of maximum and minimum temperature and wind, and finally the difference between these two which is presumed to be the measure of the population changes.

The series of values so obtained are shown graphically by the vertical solid lines in fig. 24.

It will be seen that they suggest that for the first month (March 1933, just discussed in detail) the population was almost normal; for the next two months

it was above the normal for that time of the year; and then followed a long period of seventeen months in which the population continued below normal. This spell lasted from June 1933 to September 1934, and, as will be seen by



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FIG. 24.—Mean log. population departure from the normal in the 48 months. Solid line calculated by correcting the mean log. catch for each month for the departure of the weather conditions from the normal. Dotted line forecasted from the weather conditions of previous months by regression formulae.

comparison with Table 2 (p. 231), it corresponded with a long drought which started in June 1933 with the rainfall 1.90 inches below the normal and continued until July 1934. August 1934 was the first month with the rainfall above normal (+ 0.74) and by October the population had returned to normal.

TABLE 47.

Data for the correction of the mean log. catch for any one month for the departure of the weather conditions of that month from the normal.

	Observed departure of mean log. catch from mean of same month in all four years (a)				Expected activity effect of maximum and minimum temperature and wind (b)				Difference between observed and calculated (a - b)			
	1933	1934	1935	1936	1933	1934	1935	1936	1933	1934	1935	1936
Winter :												
March . . .	+ 0.09	- 0.32	+ 0.25	- 0.01	+ 0.12	- 0.29	- 0.01	+ 0.16	- 0.03	- 0.03	+ 0.26	- 0.17
April . . .	+ 0.23	- 0.09	± 0	- 0.13	+ 0.12	+ 0.13	- 0.01	- 0.24	+ 0.11	- 0.22	+ 0.01	+ 0.11
Summer :												
May . . .	+ 0.46	- 0.31	- 0.18	+ 0.02	+ 0.26	- 0.04	- 0.30	+ 0.08	+ 0.20	- 0.27	+ 0.12	- 0.06
June . . .	- 0.15	- 0.15	+ 0.20	+ 0.09	+ 0.02	+ 0.04	- 0.01	+ 0.09	- 0.17	- 0.19	+ 0.21	± 0
July . . .	- 0.12	- 0.08	+ 0.29	- 0.08	+ 0.12	+ 0.08	+ 0.04	- 0.26	- 0.24	- 0.16	+ 0.25	+ 0.18
August . . .	- 0.28	- 0.50	+ 0.25	+ 0.57	+ 0.15	- 0.21	+ 0.10	- 0.03	- 0.41	- 0.29	+ 0.15	+ 0.48
September .	- 0.20	- 0.22	- 0.02	+ 0.42	+ 0.10	+ 0.01	- 0.18	+ 0.08	- 0.30	- 0.23	+ 0.16	+ 0.34
October . .	- 0.20	+ 0.13	+ 0.10	- 0.02	+ 0.09	+ 0.11	- 0.10	- 0.10	- 0.29	+ 0.02	+ 0.20	+ 0.08
Winter :												
November .	+ 0.01	+ 0.28	- 0.17	- 0.11	+ 0.01	+ 0.16	+ 0.01	- 0.17	± 0	+ 0.12	- 0.18	+ 0.06
December .	- 0.53	+ 0.84	- 0.22	- 0.08	- 0.41	+ 0.56	- 0.19	+ 0.04	- 0.12	+ 0.27	- 0.03	- 0.12
January . .	- 0.46	+ 0.38	- 0.02	+ 0.10	- 0.12	+ 0.13	- 0.07	+ 0.07	- 0.34	+ 0.25	+ 0.04	+ 0.03
February .	- 0.42	+ 0.20	- 0.21	+ 0.42	- 0.11	+ 0.16	- 0.26	+ 0.21	- 0.31	+ 0.05	+ 0.05	+ 0.21

It is recognised of course that "normal" is the mean of the four years and is not necessarily the average conditions for the district for the long period, but it is necessary to use this value for the meteorological conditions as I have only these four years from which to calculate the trap departures.

The population throughout the winter of 1934 to 1935 and the summer of 1935 remained above normal but after that the changes are irregular.

Forecasting.

If the above reasoning is correct, there is for 48 consecutive months a measure of the log. (or percentage) departures of the population from the normal, as given in the last four columns of Table 47; also there is a strong indication that rainfall is an important factor in determining this, although it was found to be of little importance in determining activity.

I can now apply the same statistical process of partial regressions to find out how far these population changes can be accounted for by changes in the weather conditions in the period preceding the catch.

This has been done for the effect of the two factors minimum temperature and rainfall, for each of the three preceding months on the population departure for each month. The data for this are given in Tables 47 and 48. Thus, for example, the population departure for July 1933 is - 0.24 (Table 47). The minimum temperatures in the three previous months of June, May and April were - 0.6, + 2.0 and + 1.1, and the rainfall departures - 1.90, + 0.36 and - 0.89 respectively (Table 48).

A preliminary calculation showed that there was considerable difference of effect between the winter and summer, so that finally separate calculations were made for the six "winter" months, November to April, and the six "summer" months, May to October. It would have undoubtedly been better if regressions could have been taken for shorter periods still, but even 24 months is a small number of repetitions on which to get anything like a reliable estimate of the effect of six "independent variables."

The resulting regressions for winter and summer are shown in Table 49. It will be seen that in the winter months the changes in population are best explained on the assumption that 1° F. change in the mean minimum temperature of the previous month is associated with a change of 0.0456 in the log. of the "population." A similar change in the mean minimum temperature of two months previous produces an effect of only one-third of this, while in three

TABLE 48.

Departures of minimum temperature and wind from the normal in the 48 trap months and predictions of the catch departure (and hence the population) calculated from these data.

	Departure of minimum temperature from mean of same month in 4 years				Departure of rainfall from mean of same month in 4 years (in inches)				Dept. of population predicted from regressions on minimum temperature and rainfall in 3 months previous to catch			
	1933	1934	1935	1936	1933	1934	1935	1936	1933	1934	1935	1936
Winter :												
March . . .	+0.1	-2.5	± 0	+2.2	+0.98	+0.63	-1.16	-0.46	-0.15	-0.16	+0.09	-0.11
April . . .	+1.1	+0.2	+0.6	-2.0	-0.89	-0.40	+1.82	-0.53	+0.04	-0.15	-0.02	+0.08
Summer :												
May . . .	+2.0	-0.4	-2.0	+0.2	+0.36	-0.30	+0.62	-0.69	-0.04	-0.14	+0.12	-0.10
June . . .	-0.6	-0.5	+0.8	+0.4	-1.90	-1.23	-0.08	+3.20	+0.04	-0.05	+0.22	-0.13
July . . .	+1.5	-0.5	-0.4	-0.8	-0.63	-0.91	-1.06	+2.60	-0.17	-0.16	+0.18	+0.15
August . . .	+1.7	-1.5	± 0	-0.2	-0.47	+0.74	+0.47	-0.74	-0.25	-0.23	-0.08	+0.56
September . .	+0.8	-0.8	-1.3	+1.4	-0.84	-0.46	+1.07	+0.22	-0.28	-0.09	-0.07	+0.45
October . . .	+1.0	+1.5	-0.8	+1.7	-0.57	-0.02	+0.87	-0.27	-0.17	-0.13	+0.10	+0.08
Winter :												
November . .	+0.6	+0.8	+1.0	+2.3	-1.63	-1.17	+2.09	+0.71	+0.01	+0.09	-0.05	-0.07
December . .	-5.2	+7.3	-1.9	± 0	-2.11	+2.28	+0.30	-0.47	-0.01	+0.05	+0.09	-0.14
January . . .	-2.0	+1.4	-0.5	+1.1	-0.79	-1.90	+1.14	+1.55	-0.29	+0.34	-0.06	-0.02
February . .	-3.0	+3.0	-3.0	+3.1	-1.80	+0.41	-0.12	+1.51	-0.23	+0.14	-0.01	+0.10
Minimum temperature departure					Dec. 1932		Jan. 1933		Feb. 1933			
Rainfall departure					+0.15		-0.38		-0.4			
					-1.96		-0.89		-0.44			

months previous it produces a negative effect (not significant). Rainfall, on the other hand, produces only much smaller effects varying from 0.007 to 0.019 per inch per month, the more distant month having the larger effect.

It must be noted that the variation in minimum temperature departure, from +7.3 to -5.2, during the 24 winter months is nearly three times as great as the variation in rainfall, from +2.28 to -2.11. So that the total effect of temperature is very much greater than that of rainfall.

TABLE 49.

Partial regressions of log. catch departure (after correction for activity) on the minimum temperature and the rainfall in the three previous months.

	24 Winter months		24 Summer months	
	6 factors	3 factors	6 factors	3 factors
Minimum temperature—				
month previous .	+0.0456	+0.0508 ± 0.0101	-0.0237	—
month two previous . .	+0.0151	+0.0135 ± 0.0097	-0.0003	—
month three previous . .	-0.0138	-0.0130 ± 0.0105	+0.0135	—
Rainfall—				
month previous .	+0.0101	—	+0.0916	+0.0910 ± 0.0234
month two previous . .	+0.0071	—	+0.1079	+0.1156 ± 0.0231
month three previous . .	+0.0194	—	+0.0696	+0.0645 ± 0.0224

In the summer months the values are very different. In fact the regression on temperatures is apparently negative (but not significantly so) for both previous and pre-previous months, but small and positive for three months previous. The rainfall, on the other hand, is of major importance and all three rainfall regressions are high. In addition to this it should be noted that

the variability of the rainfall in summer remains high, while that of temperature is much smaller than in winter. In the 48 summer months the minimum temperature departure only varied from $+2.0$ to -2.0 degrees; while the rainfall varies from $+3.20$ to -1.90 : a greater range than the temperatures.

Thus it appears that in the winter the population changes are chiefly associated with temperature changes in the previous three months, while in the summer the effect of temperature is almost negligible but there is a high positive relation with rainfall.

This result is a natural one, as in the Harpenden climate rainfall is evenly distributed throughout the year, but there are large changes of minimum temperature. In the summer the temperature is high for the amount of rainfall, while in the winter it is low. Thus it is reasonable that the rainfall should be the limiting factor in the summer and the temperature in the winter.

From these regressions and the known departures of temperature and rainfall an estimate can be made of the population departures for each month. This has been done and the values indicated in the last four columns of Table 48, and also diagrammatically by the dotted lines in fig. 24.

Thus there are in fig. 24 two measures of the population: (1) the solid line which is calculated from the trap catch by correcting this for activity caused by weather conditions during the particular month (Table 47 last four columns); and (2) the dotted line which is calculated from the weather conditions of the three previous months (Table 48 last four columns) and does not bring in either the trap catch or the weather of the current month. It will be seen that on the whole there is a very close resemblance between the two.

If a longer series of results had been available separate regressions could have been calculated for shorter periods—say, spring, summer, autumn and winter, and a still closer agreement might have been expected.

With the two calculations, one for summer and one for winter, and the six partial regressions analysis of variance is as follows:—

	Degrees of freedom	Total variance	Mean variance
Summer :			
Total	23	1.3382	0.0583
	6	1.0241	
Residual	17	0.3141	0.0185 = 32%
Winter :			
Total	23	0.6498	0.0283
	6	0.4212	
Residual	17	0.2286	0.0134 = 47%

Thus for the winter months 53% of the mean variance is explained by the six factors and in the summer months 68%.

As it appears that a very small amount of variance is accounted for in winter by rainfall and in summer by temperature a second set of regressions were calculated on the rainfall in the three previous months for the summer and the temperature in the three previous months in the winter. These are shown in Table 49. They differ only slightly from the previous regressions.

The analysis of variance for these three factors only is as follows:—

	Degrees of freedom	Total variance	Mean variance
Summer :			
Total	23	1.3382	0.0583
	3	0.9765	
Residual	20	0.3617	0.0181 = 31%
Winter :			
Total	23	0.6498	0.0283
	3	0.4008	
Residual	20	0.2490	0.0125 = 49%

Thus the residual mean variance is actually slightly smaller in both summer and winter when only three factors are used.

FUTURE WORK.

The results described above have been based on four years continuous trapping of insects with a single light trap, at Harpenden about 25 miles north of London. Nearly all the calculations have been made with the total catch of all insects.

If conditions permit of the experiments being continued and extended the following suggestions are made.

1. The results should be applied as soon as possible to smaller groups and particularly to individual species, and perhaps even to separate sexes of one insect. To do this with satisfactory results the number of insects caught must be greatly increased.

2. The work, whenever it is done, should be carried out with at least four traps working simultaneously under as far as possible similar conditions. This will give larger numbers of a single species and reduce the sampling error.

3. It is most important that the work should some day be repeated in a different locality, and preferably in a different climate. A series of trap catches from a tropical climate, when temperature is not the most important factor, would be of particular interest.

4. Any species of insect chosen for investigation should have the following characteristics :—

- a. It should be readily attracted to light;
- b. It should be abundant at times;
- c. It should have a very long brood or a series of broods during the year;
- d. It should be very easily distinguished from any other insect liable to be caught in the trap;
- e. If possible the sexes should be easily distinguishable.

Among insects with which I have had personal experience the Trinidad Sugar Cane Frog hopper (*Tomaspis saccharina*) appears to be a very suitable insect as it has 2 to 4 broods a year and the males come to light in very large numbers.

In this country analysis is already proceeding on about half a dozen species of moths which came to the present trap in large numbers, but the general result is to indicate that unless at least 1000 individuals are caught in a single brood the error is far too large to give any reliable results.

ACKNOWLEDGEMENTS.

Full acknowledgements have already been made in Part I of this paper and no additional names need be mentioned here. Perhaps, however, it would be as well to state that although I have received very great help and encouragement from the statistical department at Rothamsted, they are not responsible for the general argument or for any mistakes that may appear therein. I must take full responsibility for these and for the fact that I have used in analysis some less orthodox methods of demonstration because I believed them to be easier for the general entomologist to understand.

SUMMARY.

The analysis is based on four years captures in a light trap at Rothamsted Experimental Station. The trap was working on 1407 nights between March 1933 and February 1937 and caught altogether about 850,000 insects.

An account is given of the normal weather conditions in the district and also of the weather during the four trap years.

The sources of error were reduced as far as possible, and it was found necessary to deal with changes in the catch in geometrical or logarithmic proportion.

The catch is dependent chiefly on the two factors of activity and population, and, of course, only measures the positively phototropic nocturnal insects.

The analysis of the individual effect of each weather factor is made more difficult by the close correlation which exists between many of the weather factors themselves.

The first analysis was a simple comparison of the weather conditions on nights of high and low catch. On the good nights the minimum and the grass minimum temperatures of that night and the maximum temperatures of the previous day were all higher than on the poor nights; the wind was also calmer; the moon closer to new moon than to full, and the barometer high. Rain during the previous daytime is associated with low catch, but rain during the night occurs with equal frequency with high as with low catch. Relative humidity also appears to be more or less similar in both series.

A short account is then given of the statistical method of regressions and it is shown that activity is most likely to be correctly estimated by using as a basis of calculation the difference between successive days in both catch and weather conditions. The effect of minimum temperatures, considered alone, is that a change of 1° F. is associated with a change of log. catch of about 0.060, or, in other words, the catch is doubled by an increase in minimum temperature of 5° F. (or just under 3° C.). There is no evidence from the data available that this figure alters with the season, the differences found from month to month are irregular and not outside the limits of non-significant variation. Results for Lepidoptera only and Hemiptera only give values which do not differ significantly from the value for total insects.

The effect of maximum temperature alone is smaller than that of minimum temperature and the average effect (over the 4 years) is that 1° F. change is associated with 0.042 change in log. catch, or the catch is doubled with a rise of about 7° F.

The grass minimum alone (calculated from 6 months results) shows a regression of 0.030. The regression on daily range of temperature (from 3 months) is only—0.004, which has no significance.

Relative humidity at 9 p.m. shows a small but probably significant regression of about 0.008 change in catch produced by 1% change in humidity.

Insects fly later on cloudy nights, probably owing to the slow fall in temperature. The effect of fog is uncertain; usually it occurs on cold nights with very poor catch, but one very foggy night in September 1933 gave high catches in the family NOCTUIDÆ.

The catch is lower at full moon than at new moon and there are asymmetries in the effect that can be explained by similar asymmetries in the times of rising and setting of the moon.

Wind force was divided into six artificial groups, the lowest being dead calm and the highest wind over 20 m.p.h. The effect is complicated by the

fact that the windy nights are warmer than the still nights, particularly in the winter. When the method of difference between successive days is used the effect of a change of one group was to alter the log. catch by 0.095 (other factors being neglected).

The effect of barometric pressure is complicated and difficult to understand. Catch is low with low barometer, and high with high barometer unless pressure is falling; in this latter case the catch is distinctly lower. If the direction of movement is neglected the average effect of a rise of 1 in. in the barometer is to add 0.668 to the log. catch. The catch is therefore doubled by a rise of 0.48 inches ($= 1.14$ cms.).

There is no evidence of the effect of thunderstorms on the total catch of all insects, but slight indication that moths of the family NOCTUIDAE are more abundant during thundery weather.

The simultaneous analysis of several factors acting together show differences from some of the above results owing to correlation between the weather conditions themselves. Analysis of 9 p.m. temperature and humidity, expressed as Relative Humidity, Absolute Humidity and Saturation Deficiency, shows no advantage to be gained by using one method rather than another.

With partial regressions of catch on the minimum temperature of several nights preceding the catch, it was found that the previous night has about $\frac{1}{2}$ of the effect of the current night, but the night before that had on an average no effect.

When maximum and minimum temperatures and wind were taken simultaneously the regressions of each were 0.023; 0.070 and 0.144 respectively.

For the year 1933 the simultaneous effect of the above three factors plus 9 p.m. relative humidity was calculated and the regressions were 0.016; 0.065; 0.121 and 0.006.

It is possible to correct the mean catch for each month for the effect of its departures from the normal in temperatures, wind, etc., thus getting a value for what the catch would have been if all conditions had been normal for the month. The differences then remaining are due to population effects. These values have been calculated for the 48 months in which the trap was running.

The relation of these values to the rainfall and minimum temperature of the three previous months has been calculated and two regression formulae obtained, one for winter and one for summer, from which the population changes can be estimated from a knowledge of previous weather conditions only. Rainfall is most important in summer and temperature in winter.

The population changes as estimated from the trap catch and as calculated from the rainfall and temperature in the three previous months are shown in fig. 24, and it will be seen that they are closely similar. Between 50% and 60% of the variance of the population has been accounted for.

Thus a beginning has been made in the problem of measuring the variations in abundance of insects and in forecasting this from a knowledge of the weather conditions. So far it has only been described for the total population of all insects but it will later be extended to single species.

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STUDIES OF FLUCTUATIONS IN INSECT POPULATIONS

VII. THE BUTTON TOP MIDGE (*RHABDOPHAGA HETEROBIA*)
AT SYSTON, 1934-39

BY H. F. BARNES

*Entomology Department, Rothamsted Experimental Station**(With 4 Figures in the Text)*

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1. INTRODUCTION

THIS is the seventh of a series of papers giving the results of an attempt to study fluctuations in insect populations as they occur in nature. The first five of these studies, all of which were concerned with various species of gall midges, have been discussed in the sixth of the series (2) and another paper (3).

The present contribution is a continuation of the account, started in the third paper of the series (1), of the "button-top" midge, *Rhabdophaga heterobia* H.Lw. (Diptera, Cecidomyiidae), on *Salix triandra* variety Black Maul at Syston, Leicestershire. That paper covered the years 1928-33, while the present one deals with 1934-9. In the last section the results of the whole twelve-year period have been discussed. The present paper should be read in conjunction with the general account of the pests on the willows at Syston which was written by Roebuck (4), together with the third (1) and sixth (2) papers in the present series.

The methods used have been the same as in the previous years. The standard size of sample throughout has been 500 galls.

Unfortunately, the particular field of willows, from which the material has been collected each year, has been allowed to go out of cultivation since 1936. The method of eradicating the willows has been to cut down the annual growth at the end of each winter and then allow horses and cattle to graze during the summer. These animals have kept on eating the willow shoots with the result that the annual growth has become less and less until, in the autumn of 1938, the relatively few willows that had survived such treatment were scarcely alive

and the year's growth only a foot or so in height. "Button" galls, however, were still present on almost 100% of the shoots, but they were noticeably small. By the autumn of 1939 the willows had for all practical purposes been exterminated and the field study perforce had to be drawn to a conclusion.

2. SIZE OF POPULATION OF *RHABDOPHAGA HETEROBIA* AND ITS PARASITES, 1934-9

The average numbers of the gall midge and its parasites reared are expressed diagrammatically in Fig. 1. Table 1 gives the numbers of midges and Hymenopterous parasites reared over the period of 1934-9. Both this figure and table are continuations of the corresponding figure (Fig. 1) and table (Table 4) in the third paper of this series (1). The figure for the year in each case denotes the year in which the insects emerged: the samples were collected the previous

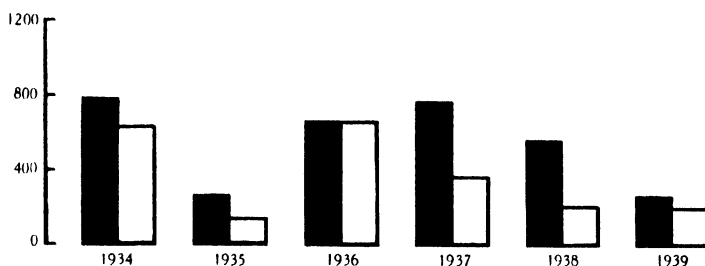


Fig. 1. Average numbers of *Rhabdophaga heterobia* (solid) and parasites (open) emerging from 500 galls, 1934-9.

autumn between 30 October and 2 November. Sample IV, from which insects emerged in 1936, is the exception. This sample was collected on 24 January 1936, whereas the other three samples emerging that year were collected at the normal time the previous year, i.e. 30 October.

This late collection of material was made in order to compare the population of the galls present in January with that of galls present at the end of October. What effect would the winter and consequent hunting for food by birds have had on the insect population? As soon as the field was visited in January, it was obvious that birds had been diligent in eating out many of the larger and more obvious galls. In fact it was difficult to find any of normal size, those remaining being all undersized as compared with those of the previous October. Table 1 shows that the total insect population was slightly over 900 compared with 1200-1500 of the samples collected before the winter months. Both the numbers of the midges and parasites, as to be expected, were reduced considerably. In other respects, i.e. dates and crests of emergence of host midge and its parasites (D, Table 3) sex ratio of the midge and relative parasitism, the samples collected in October and January did not appreciably differ.

The successive reduction in total insect population from 1933 (average 3238 midges and parasites) to 1934 (average 1429 midges and parasites) and to

1935 (average 382 midges and parasites) may have been due, partially at any rate, to the effect of the comparatively dry hot summers of 1933 and 1934 and the consequent diminution of luxuriant willow growth. It must be remembered that only the overwintering generation of midges and parasites was studied, and so the 1934 population recorded would be that which developed in the late summer of 1933. In 1936, i.e. after the summer conditions of 1935, the population had risen again to an average total of 1350, still less than half the 3000 estimated (1) normal insect population for 500 galls. In 1937 the effect of allowing the willows to go out of cultivation prevented any further return to the normal and, subsequently, in 1938 and 1939 this effect was overwhelming, the average population dropping from 1129 in 1937 to 786 and 742 in 1938 and 1939 respectively.

Table 1. *Population of 500 galls of Rhabdophaga heterobia*, 1934-9

Year	Total midges	Sex ratio	Total Hymenopterous parasites	Parasitism %	Total insects
1934 (i)	876	44 : 56	566	39	1442
(ii)	858	43 : 57	647	43	1505
(iii)	655	49 : 51	687	51	1342
1935 (i)	245	41 : 59	147	37.5	392
(ii)	253	40 : 60	158	38	411
(iii)	203	35 : 65	139	40	342
1936 (i)	637	43 : 57	601	48.5	1238
(ii)	590	49 : 51	671	53	1261
(iii)	799	45 : 55	751	48	1550
* (iv)	416	44 : 56	520	55.5	936
1937 (i)	790	41 : 59	288	27	1078
(ii)	788	43 : 57	410	34	1198
(iii)	772	41 : 59	340	31	1112
1938 (i)	566	39 : 61	191	25	757
(ii)	543	41 : 59	226	29	769
(iii)	614	48 : 52	219	26	833
1939 (i)	261	44 : 56	207	44	468
(ii)	216	34 : 66	222	51	437
(iii)	320	43 : 57	191	37	511

* Galls collected on 24 January 1936.

The sex ratio of the midge during the period under consideration varied from 34 : 66 to 49 : 51 which closely resembled the range (35 : 65 to 48 : 52) during the previous six years. There was no evidence that the numbers of males decreased as the total midge population increased as had been previously suggested.

Three further examples of hermaphrodite individuals have been reared; one in 1934 which had male antennae but possessed an ovipositor, one in 1936 with female antennae and male genitalia and one in 1938 also with female antennae and male genitalia. Out of about 30,000 midges reared in the 12 years, there have been only 5 such individuals, of which four had female antennae and male genitalia.

3. RELATIVE PARASITISM OF THE MIDGE

The parasites involved comprise six species (1). All the parasites reared during the twelve years, 1928-39, have been retained; those emerging in the seven years 1928-32 and 1938-9 have been kept according to which sample they belonged irrespective of their dates of emergence, while those emerging during the five years 1933-7 have been kept according to the dates of emergence irrespective of the particular sample from which they emerged. A start has

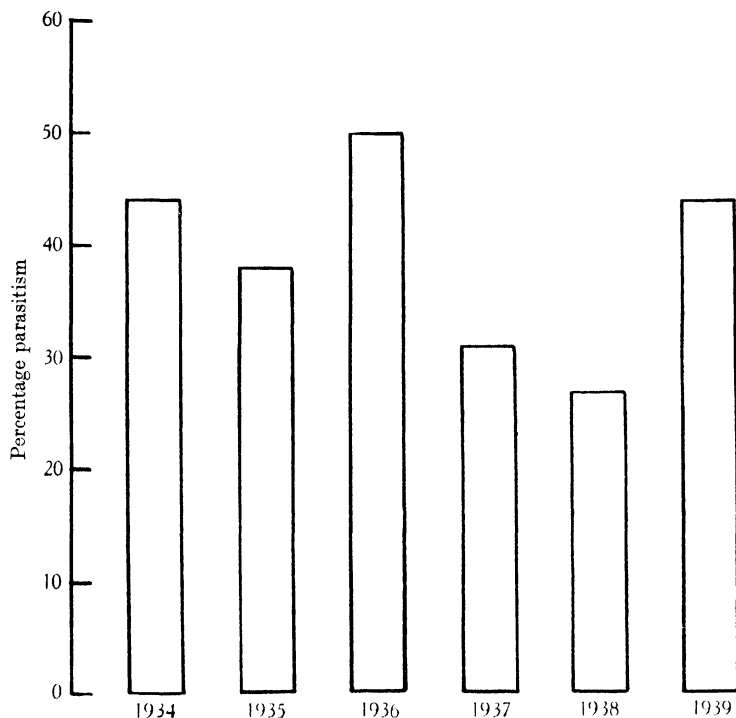


Fig. 2. Average relative parasitism of overwintering generation of *Rhabdophaga heterobia*, 1934-9.

been made in studying the numerical interrelationships of the various species and their seasonal emergence, and it is hoped to publish the results in due course, and so conclude this study of *R. heterobia* and its parasites.

Meanwhile, the gross relative parasitism in the separate samples can be seen in Table 1, and the average yearly relative parasitism of the midge is set out graphically in Fig. 2. This figure is the continuation of the corresponding figure (Fig. 2) in the previous paper (1). On the whole, the degree of parasitism has been less than in the previous six years, excluding the peculiar year 1933. Although the average total numbers of midges and parasites fell from 3238 in 1933 to 1429 in 1934 and to 382 in 1935 (Table 4) the average relative parasitism did not depart very appreciably from the 50% level. This would support

the suggestion made in the previous section that the reduction in total population was perhaps due to the effect of the summers of 1933 and 1934 on the willows' growth. From 1936 to 1938 there was a decrease in relative parasitism. This decline is probably partially due to the effect of the willows being allowed to go out of cultivation, although the rise in relative parasitism in 1939 would seem to indicate that the availability of midges as food for the parasite complex plays a not inconsiderable part (Fig. 3).

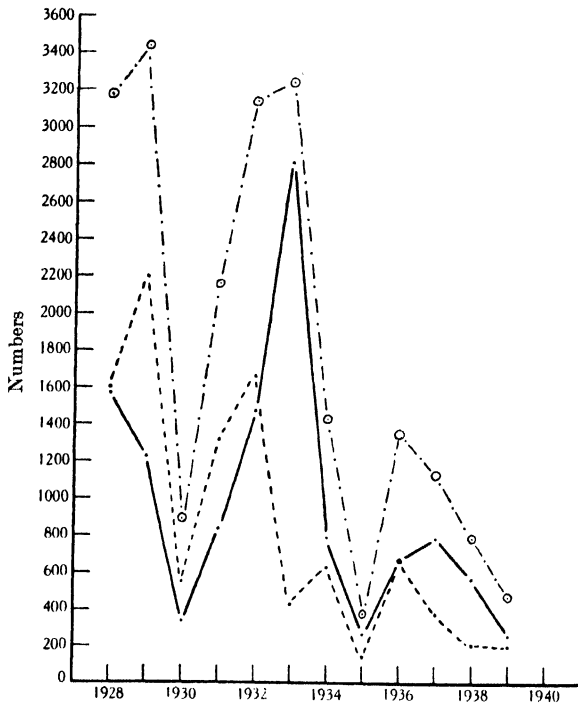


Fig. 3. Average total numbers of *Rhabdophaga heterobia* plus its parasites (— · — · —); average numbers of gall midges alone (—) and of parasites alone (---); for years 1928–39.

4. EMERGENCE

Table 2 (compare Table 5 in (1)) shows the dates of the actual first emergence, the peak of emergence, the number of days to reach the peak and the date of the last emergence of *R. heterobia* in the samples as kept in the unheated insectary during the period 1934–39 inclusive.

The range of first emergence was from 13 April to 12 May (29 days), while that of the peaks is from 19 May to 5 June (17 days). Within samples of the same year the corresponding range between dates of first emergences has been 4 days in 1935 and 1939, 3 in 1934, 2 in 1937 and 1 day in 1936 and 1938. In the last-named year in samples (ii) and (iii) only single individuals emerged on 13 and 28 April and emergence did not start again till 4 and 5 May. The

time which lapsed between the first emergence and the peak varies from 10 to 32 days if one ignores the second sample of 1939 for the reason given above. The variation within any one year is less, 14–15 days in 1936, 22–24 days in 1937, 21–25 days in 1939, 23–30 days in 1935, 22–32 days in 1938 and 10–24 days in 1934.

Table 2. *Dates of actual first emergence, peak of emergence, number of days to reach peak and the dates of the last emergences of Rhabdophaga heterobia, 1934–9*

Year	Date of first emergence	Date of peak of emergence	Days to reach the peak	Date of last emergence
1934 (i)	9 May	19 May	10	1 July
(ii)	10 May	3 June	24	3 July
(iii)	12 May	23 May	11	4 July
1935 (i)	5 May	30 May	25	9 July
(ii)	7 May	30 May	23	5 July
(iii)	3 May	2 June	30	5 July
1936 (i)	5 May	19 May	14	1 July
(ii)	4 May	19 May	15	27 June
(iii)	5 May	19 May	14	30 June
1937 (i)	7 May	29 May	22	13 July
(ii)	5 May	29 May	24	7 July
(iii)	5 May	29 May	24	5 July
1938 (i)	4 May	5 June	32	20 July
(ii)	13 April*	5 June	53 (32)	29 June
(iii)	28 April*	27 May	29 (22)	6 July
1939 (i)	7 May	30 May	23	19 July
(ii)	6 May	31 May	25	9 July
(iii)	10 May	31 May	21	10 July

* Single individuals, then (ii) restarted on 4 May and (iii) on 5 May.

The actual peak day of emergence, however, is not so reliable as the week in which the maximum numbers of midges (or parasites) emerged. The dates and figures in Table 3 (corresponds to Table 6 in (1)) shows the weekly emergences of the midge and its parasites. The top row of figures in each case refers to the midge, while the lower row refers to the parasites. The peaks are in heavy type. It will be seen that a weekly crest of emergence of the midge has occurred three times in the week 14–20 May, four times in the week 21–27 May, ten times in the week 28 May–3 June and twice in the week 4–10 June. However, these latter two occurrences might well have been in the week 21–27 May. In the three samples of any one year the peak has occurred two years in the same week, three years within a fortnight and one year in a period of three weeks.

The peak emergence of the parasites has occurred three times in the week 28 May to 3 June, nine times in the week 4–10 June, three times in the week 11–17 June and three times in the week 25 June to 1 July. As in the previous study, no variation occurred within the samples of any one year. Usually the peak week of emergence of parasites occurred in the first to fourth week after that of its host midge; but in 1937 and 1938 the weekly peak of the parasite was the same as that of the midges in five out of six samples.

Table 3. *Weekly emergence of Rhabdophaga heterobia and its parasites, 1934-9. The upper rows of figures refer to the midges while the lower refer to the parasites. The figures in heavy type indicate the peak weeks*

Year	Date and size of sample	23-29 April	30 April	7-13 May	14-20 May	21-27 May	28 May	4-10 June	11-17 June	18-24 June	25 June	2-8 July	9-15 July	16-22 July	July-August later	Total parasites	Para-host and stism %
1934	31 Oct. 1933, 500 galls, A	—	—	73	153	270	273	76	22	4	5	—	—	—	—	876	39
		—	—	1	0	12	102	154	203	63	18	10	2	1	—	566	36
	B	—	—	20	84	239	314	110	65	17	8	1	—	—	—	858	43
	C	—	—	9	61	239	193	73	41	18	14	17	—	—	—	647	51
		—	—	—	—	15	141	196	227	71	20	10	6	0	1	687	51
1935	1 Nov. 1934, 500 galls, A	—	3	14	19	56	56	31	23	14	27	1	1	—	—	245	37.5
		—	—	—	1	2	8	8	15	37	42	27	7	—	—	147	—
	B	—	—	15	17	54	68	60	20	7	11	1	—	—	—	253	38
	C	—	4	8	5	34	42	16	17	42	48	16	2	1	—	158	—
		—	—	—	—	—	—	37	29	19	21	3	1	—	—	203	40
1936	30 Oct. 1935, 500 galls, A	—	1	3	193	241	103	92	16	13	5	—	—	—	—	139	—
		—	—	19	67	81	81	190	144	98	2	—	—	—	—	637	48.5
	B	—	2	10	230	139	68	104	28	8	1	—	—	—	—	601	—
	C	—	1	17	25	103	125	212	129	74	2	1	—	—	—	590	53
		—	—	—	248	216	103	140	61	12	1	—	—	—	—	671	—
24 Jan. 1936, 500 galls, D		—	—	4	159	101	54	223	187	87	3	1	—	—	—	799	48
		—	—	—	31	122	117	66	15	16	1	—	—	—	—	751	—
		—	—	—	6	40	72	172	155	72	1	1	—	—	—	416	55.5
1937	30 Oct. 1936, 500 galls, A	—	—	3	32	180	289	217	42	14	10	2	1	—	—	790	27
		—	—	—	30	30	120	63	47	17	9	2	—	—	—	288	—
	B	—	1	9	36	195	301	170	55	11	6	4	—	—	—	788	34
	C	—	4	7	42	213	166	118	45	27	11	2	—	—	—	410	—
		—	—	—	42	213	319	138	26	13	9	1	—	—	—	772	31
		—	—	—	29	153	453	97	33	12	15	1	—	—	—	340	—
1938	2 Nov. 1937, 500 galls, A	—	2	20	90	156	75	161	45	13	3	0	0	1	—	566	25
		—	—	—	3	33	32	64	35	20	0	2	0	2	—	191	—
	B	1	3	29	82	135	113	143	27	6	4	—	—	—	—	543	29
	(13 April)	—	—	—	6	26	42	80	40	24	7	0	0	1	—	226	—
	C	1	2	7	96	175	121	148	49	8	4	3	—	—	—	614	26
		—	—	—	3	17	27	58	57	45	10	2	—	—	—	219	—
1939	31 Oct. 1938, 500 galls, A	—	—	8	14	93	82	37	12	5	5	3	1	1	—	261	44
		—	—	—	9	9	52	105	25	12	3	1	—	—	—	207	—
	B	—	1	5	4	53	69	47	16	6	12	2	1	—	—	216	51
	C	—	—	8	19	87	121	94	38	13	1	3	0	1	—	222	—
		—	—	—	—	—	—	56	12	4	5	6	2	—	—	320	37
		—	—	—	5	49	49	91	28	14	3	1	—	—	—	191	—

An additional sample of 500 galls collected on 1 November 1935 was placed in a warm glasshouse on 5 March 1936 after being previously kept in the usual unheated insectary. The midges and parasites quickly responded to the extra warmth. The former started emerging on 19 March compared with 4 May in the samples kept continuously in the insectary. The parasites started on 23 March as compared with 15 May. The crest of emergence of the midges was on 3 April. The emergences of midges ended on 1 May, while that of the parasites ended on 30 April. The duration of the emergences was distinctly shorter than under the insectary conditions of normal outside varying temperatures. Under these conditions of extra heat in the spring the duration of midge emergence was 43 days compared with 57, 54 and 56 days under normal conditions. The crest was reached after the same number of days, under the extra heat (15 days) and under normal temperatures 14, 15 and 14 days) (see Table 2). Slightly fewer midges and parasites (475 and 595) emerged under these glasshouse conditions than under the usual insectary ones (637, 590, 799 midges and 601, 671 and 751 parasites). The parasitism (55.6%) and the sex ratio (46:54) showed no appreciable difference.

5. DISCUSSION OF RESULTS, 1928-39

The particular field (planted in 1918) in which the galls were collected each year was chosen because it formed part of an old willow bed (established for over 40 years). Ample time should have lapsed since its original planting for the button-top midge and all its parasites and predators to have arrived and become firmly established. It has already been pointed out (2) that the weather may react directly on the host insect (*R. heterobia*) or indirectly on it through its effect on the host plant or on the insect's parasites and other enemies.

It is convenient to consider the results under three headings, viz. (a) the insect population (i.e. the host gall midge and its parasites) of 500 galls, (b) the relative parasitism of the midge (i.e. the proportion of parasites -midges emerging) and (c) the emergence of the gall midge. A discussion of the emergence of its parasites and the relative numbers of the various species involved must be left to a further paper.

It must be remembered throughout that only the overwintering generation, i.e. every third, was studied.

(a) *Insect population of 500 galls*

Fig. 3 shows graphically the average total numbers of the gall midge and its parasites (i.e. the total number of midges if there had been no parasitism), the average totals of the gall midge alone and the average totals of all its parasites considered together for the whole period under discussion. Table 4 gives the details and the average relative parasitism in addition. In four years (1928, 1929, 1932 and 1933) the total insect population was slightly over 3000.

It is thought that under ordinary good conditions of plant growth this figure represents the insect population that can be maintained in 500 galls. This number is not the number which would normally reach the adult state owing to the fact that various birds, especially tits, raid the galls during the winter months. The extent of this reduction in population can be judged by the figures given in Table 1 for the sample (D) collected in late January compared with those (A, B, C) collected at the end of the previous October. It must be remembered in this connexion, however, that tits when marauding the galls drop a considerable portion of each gall to the ground and doubtless some insects could be found in these bits. They would certainly be able to come to maturity on the ground.

Table 4. *Average numbers of midges, parasites and total insects in 500 galls, 1928-39*

Year	Midges	Parasites	Total insects	% parasitism
1928	1573	1607	3180	51
1929	1235	2204	3439	64
1930	341	556	897	62
1931	840	1323	2163	61
1932	1480	1662	3142	53
1933	2810	428	3238	13
1934	796	633	1429	44
1935	234	148	382	38
1936	675	674	1349	50
1937	783	346	1129	31
1938	574	212	786	27
1939	265	207	472	44

In 1930 and also in 1934 and 1935 the total insect population showed remarkable drops. It has already been suggested (1) that the 1930 decrease was due to the effect of the August-September 1929 drought on the behaviour of the sap in the willows. The sap stopped rising, thus cutting the food supply of the insects about to overwinter, and the winter buds were formed very early. In a similar manner the hot dry summers of 1933 and 1934 may have been responsible for the fall in insect numbers in 1934 and 1935 through their effect on plant growth. In the first instance, the willows and the insect population took two seasons 1930 and 1931 (or six generations of midge) to recover their normal growth and numbers, the latter in the spring of 1932.

In the second instance the 1936 figures showed an increase over the 1935 figures, but further recovery to normality was prevented by the fact that by this date the willows were being allowed to go out of cultivation and be exterminated by cattle. From this date onwards the insect population steadily decreased for the same reason.

(b) *Relative parasitism of the midge*

In addition to the six species of parasitic Hymenoptera that have been reared, a predaceous Cecidomyid, *Lestodiplosis* sp., has been reared in insignificant numbers as follows: 1928, 3; 1929, 18; 1930, 12; 1931, 3; 1932, 3; 1933, 4; 1934, 11; 1935, 1; 1936, nil; 1937, 1; 1938, nil; 1939, 2.

Reference to Table 4 and Fig. 3 shows that there exists a positive correlation between the numbers of midges and parasites. This suggests that relative parasitism as high as 64% exerts no control on the numbers of midges. There is no correlation between the size of total population (midges and parasites) and the percentage parasitism. Additional examination of the data involving the percentage changes from year to year in the numbers of the total population and in those of the parasites reveals that such changes are almost identical (regression of log of parasites on log of total population equals +0.9). This indicates that the parasites do not become any more efficient at high populations than at low ones and therefore cannot have any balancing effect by cutting off extreme fluctuations.

There is no evidence that the absolute number of parasites lags behind that of the host midge. This may be due to the fact that only every third generation was studied.

Fig. 3 also shows clearly that when the numbers of midges dropped in 1930 and again in 1935, the numbers of parasites also dropped, while the relative parasitism was hardly affected.

On the other hand, the relative parasitism in 1933 was exceptionally low, although the numbers of midges was high. It has previously been suggested (2) that a reversal in the relative times of emergence of host insect and parasites is a possible explanation of this sudden fall in numbers of parasites.

(c) Emergence

Table 5 shows the frequency of the weekly period of peak emergence of *R. heterobia* and its parasites, collected at Syston in the autumn and kept over the winter at Harpenden, during the twelve years under consideration. It will be seen that usually most of the midges emerged in the week 28 May to 3 June, while most of the parasites emerged within the subsequent two weeks. The details of the actual numbers of midges emerging have been given in Table 3 of the present paper and Table 6 of (1).

Table 5. *Frequency of peak emergence of R. heterobia and its parasites, 1928-39*

Week	Occurrences	
	Midge	Parasites
14-20 May	2	—
21-27 May	9	—
28 May-3 June	15	3
4-10 June	3	12
11-17 June	3	11
18-24 June	—	3
25 June-1 July	—	3

Fig. 4 gives the dates of first emergence of the midge and its parasites during the twelve years as well as the peak weeks. The full lines represent the midge, while the dotted lines represent the parasites. The horizontal lines

represent the weeks of the peak emergences. It will be seen that the seasonal effect on the first dates of midge emergence is large, the range of first emergence extending from 13 April to 16 May. But the peak week of midge emergence, i.e. the week in which the greatest numbers of the midges emerge, is fairly constant. In the case of the first emergences the parasites¹ are not so affected as the midges, but the peak weeks of the parasites are more variable than those of the midges. This is probably due to variations from year to year in the numbers of individuals in the six species of parasites involved and the seasonal dates of emergence of the species. The first dates of emergence do not appear to be such reliable indicators of the season's earliness or lateness as are the peak weeks of emergence. But it must be remembered that "earliness" or "lateness" alters very rapidly even from week to week in some seasons.

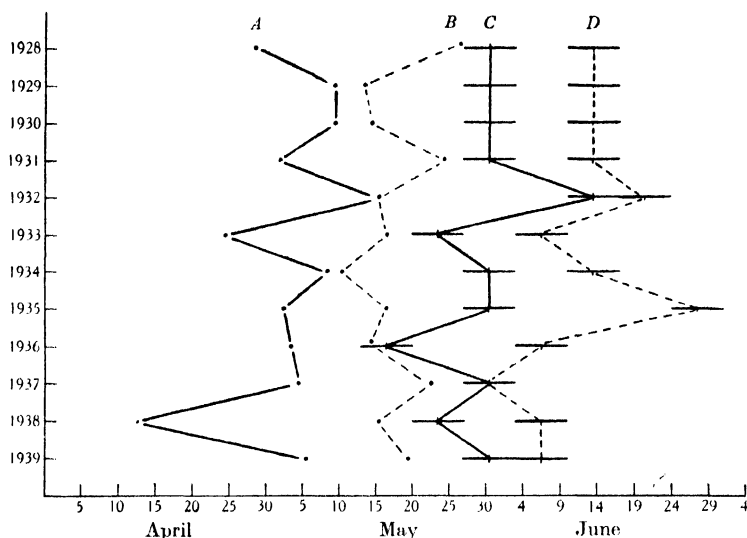


Fig. 4. First and peak weeks of emergence of *Rhabdophaga heterobia* and its parasites, 1928-39. A, 1st emergence of *R. heterobia*; B, that of parasites; C, peak week of *R. heterobia*; and D, that of parasites.

The view is put forward that the graphs indicate that although the weather usually affects the midge and its parasites to the same extent, it does not invariably do so. If this is the case one can see how in some years, although the parasites are abundant, they will not be able to attack successfully the midges owing to the different relative periods of emergence.

In conclusion, this twelve year study has served to illustrate the immense complexity of populations in the field and the almost insuperable difficulties encountered when studying them. However, this type of population study could be developed and improved in at least three ways. First, when the

¹ It is thought justifiable to consider that the first parasite to emerge will in all probability be the same species each year.

seasonal appearance and interrelationships of the various species of parasites involved have been worked out as far as possible, the part they play should be considerably clearer. Secondly, in this particular study only the overwintering generation of the midge has been under consideration. Thirdly, twelve years is only a short period during which to observe the effect of weather; a far longer period is to be desired. Data of this nature can be amassed without interrupting other work and in fact it becomes a matter of simple routine. For this reason it is to be hoped that, however indeterminate the present results appear, other workers will be encouraged to accumulate similar data on various insects and rest assured that their work will be of some use. One major difficulty, which apparently everyone will meet, is that with the exception of a few species the parasitic Hymenoptera are inadequately known, while the knowledge of their life histories lags still further behind.

6. SUMMARY

1. This study of the gall midge *Rhabdophaga heterobia* H.Lw. is a continuation of the third study in the series, which covered the years 1928-33, and the data for the years 1934-9 are given. In addition, the results of the whole twelve years, 1928-39, are reviewed.

2. The changes in the total insect population (midge and parasites) have been discussed. Birds are shown to play an important role in reducing the total population during the winter months. The drop in 1930 was probably caused by a drought in the late summer of 1929. The hot summers of 1933 and 1934, acting through the plant growth, caused similar reductions in 1934 and 1935. Since 1936 the population has steadily fallen because the willows have been allowed to go out of cultivation and become exterminated as a result of grazing.

3. A positive correlation has been found to exist between the numbers of midges and parasites, but there is no correlation between the size of the total population (midges and parasites) and the percentage parasitism. The changes from year to year in the numbers of the total population and in those of the parasites are almost identical. It is concluded that relative parasitism as high as 64% exerts no control on the number of midges and also that the parasites do not become more efficient at high population levels than at low ones and so cannot have any balancing effect.

4. The readiness of the midge and its parasites to emerge in response to extra warmth given in March has been demonstrated. Under normal conditions dates of first emergence have varied from 13 April to 16 May in the case of the midge, but the variation is less when the weeks of maximum emergences are considered. The emergence of the parasites usually follows closely that of the midge but in some years the weather appears to act differentially on the midge and its parasites as regards the dates of emergence.

7. ACKNOWLEDGEMENTS

I am deeply indebted to Mr A. Roebuck for his continued great help in collecting the material and discussing the whole problem. My thanks are also due to Dr C. B. Williams and Dr A. C. Evans for their assistance in the more statistical side of the investigation.

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STUDIES OF FLUCTUATIONS IN INSECT POPULATIONS

VIII. THE WHEAT BLOSSOM MIDGES ON BROADBALK, 1932-40,
WITH A DISCUSSION OF THE RESULTS OBTAINED 1927-40

BY H. F. BARNES

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1. INTRODUCTION

THIS is the eighth of a series of papers giving the results of an attempt to study fluctuations in insect populations as they occur in nature. The present contribution is a continuation of the account, started in the first paper (1) of the series, of the wheat blossom midges, *Contarinia tritici* Kirby and *Sitodiplosis mosellana* Géhin, on the classical field of wheat (Broadbalk) at Rothamsted Experimental Station, Harpenden.

In sections 2 and 3 new information is given covering the period 1932-40, and in section 4 the data for relative parasitism from 1928-9 to 1939-40 are laid out. In section 5 the emergence of the midges and their parasites, incidence of attack by the midges and the relative parasitism for the whole period, 1927-40, are discussed. In the remaining sections questions of more general interest receive attention.

The methods used have been basically the same as in the previous years (1927-31). However, when the plots on the field were divided into fifths and

one-fifth left fallow each year (1935 onwards), 13 ears were taken as a sample from each fifth under wheat of the ten plots considered, making a total of 52 ears from each plot. This enabled the incidence of attack on the first, second, third and fourth wheat crops after one year's fallow to be observed. But when considering the population of the field as a whole 50 ears only from each of the ten plots have been taken into account.

2. BIOLOGY

(a) Emergence

The dates of emergence of the two midges in 1929-31 were not given in the previous contribution (1) and are included in Tables 1 and 2. It will be seen that the crest of emergence (denoted by heavy type) of *C. tritici* has always occurred in the three-week period 4-24 June. In 1933 and 1940 the peak was reached earliest, i.e. 4-10 June, but in the remaining ten years, the peak was reached five times in the week 11-17 June and five times in the week 18-24 June. In the case of *S. mosellana* the range of peak emergence has been a week longer and later, 4 June-1 July. In 1933 and 1937 the crest was reached in the week 4-10 June, in four years in the week 11-17 June, in four years in the week 18-24 June and in two years 25 June-1 July.

The dates of first emergence can be ascertained by reference to Fig. 4 in section 5. In the case of *C. tritici* they have varied from 30 May in 1933 and 31 May in 1940 to 17 June in 1929. In the case of *S. mosellana* they range from 29 May in 1933 to 17 June in 1929.

Most years the above peaks of emergence obtained in the insectary were checked by observation with those actually occurring on Broadbalk. It was obvious that the correspondence was quite good. In addition, in 1935 Mr P. S. Milne placed his mechanical trap (8) in Broadbalk and the captures of both species of midge were recorded daily.

Figs. 1 and 2 show the daily numbers of *C. tritici* and *S. mosellana* respectively caught in this manner and those obtained by rearing them in the insectary. Since the midges are short-lived the numbers caught will correspond closely with those of actual emergence and oviposition. From the graphs it will be seen that the dates of the midges in the field correspond closely with those bred in the insectary. Naturally the curves of captures vary very much more from day to day than those reared under much more constant conditions. In fact they resemble in their daily fluctuations those caught in 1927 (Fig. 3 and Table 4 in the previous paper).

A comparison of the three methods—handpicking ovipositing females during 1½ hr. five evenings a week (1), capture by means of the mechanical trap running continuously and breeding in the outdoor insectary—is quite interesting. The results are shown in Table 3.

Table 1. *Weekly emergence of Contarinia tritici and its parasites, 1929-40*

Week	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938	1939	1940
Aug. previous year	—	234-0	11-0	36-0	15-0	5-0	19-0	8-0	2-0	12-0	—	Some
Sept. previous year	—	0-1	—	18-0	—	—	—	—	—	—	—	—
7-13 May	—	—	—	—	—	—	—	—	—	—	—	—
14-20	—	—	—	—	—	—	—	—	—	—	—	—
21-27	—	—	—	—	—	—	—	—	—	—	—	—
28-3 June	—	—	—	—	4-0	—	—	—	—	—	—	23-0
4-10	—	31-0	4-0	—	52-94	—	3-0	—	132-0	37-0	3-0	559-0
11-17	2-0	2092-16	632-1	221-0	0-57	3-0	18-0	176-0	168-23	435-10	59-0	0-1
18-24	150-2	411-821	225-649	1187-4	—	*(219)	591-13	1189-187	1-38	83-29	146-1	—
25-1 July	24-7	7-92	12-316	362-1190	—	6-10	302-385	13-19	0-6	3-3	32-5	—
2-8	5-5	3-4	0-13	106-358	—	0-1	1-30	—	—	—	2-0	—
9-15	0-5	—	—	34-18	—	—	0-4	—	—	—	0-0	—
16-22	—	—	—	—	—	—	—	—	—	—	0-1	—
23-29	—	—	—	—	—	—	—	—	—	—	—	—
30-5 Aug.	—	—	—	—	—	—	—	—	—	—	—	—
% emergence	16	19	12	20	3	3	41	37	52	24	66	53

* Crest of midge emergence from extra material (in brackets).

Table 2. *Weekly emergence of Sitodiplosis mosellana and its parasites, 1929-40*

Week	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938	1939	1940
7-13 May	—	—	—	—	—	—	—	—	—	—	—	—
14-20	—	—	—	—	—	—	—	—	—	—	—	—
21-27	—	—	—	—	—	—	—	—	—	—	—	—
28-3 June	—	—	—	—	15-13	—	—	—	—	—	—	—
4-10	—	1-0	2-0	—	73-346	—	—	—	58-8	1-2	50-0	41-19
11-17	1-0	94-4	32-12	5-2	12-202	3-1	4-0	59-2	43-87	83-44	432-3	143-17
18-24	23-7	21-41	15-128	18-68	11-40	(34)*	184-15	127-105	4-64	130-49	269-8	8-29
25-1 July	12-39	8-40	36-192	50-276	1-9	(14)	56-69	628-74	2-84	7-40	87-25	0-3
2-8	11-55	1-8	3-137	36-230	39-1	4-2	0-15	7-9	0-12	0-4	153-6	—
9-15	0-26	—	0-11	1-35	0-4	—	0-1	0-1	—	—	50-1	—
16-22	0-3	—	0-2	0-3	0-1	—	—	—	—	—	—	—
23-29	—	—	—	—	0-1	—	—	—	—	—	—	—
30-5 Aug.	—	—	—	—	0-1	—	—	—	—	—	—	—
% emergence	26	37	16	13	27	4	60	38	13	11	131†	16

* Midges emerging from extra material.

† For explanation see section 2 (b).

It will be realized that handpicking is the most laborious method and gives no idea of the sex ratio. Trapping also gives no true idea of the sex ratio, and the need for accurate determination of the species is involved as other species are also caught. Both handpicking and trapping, however, reveal the day-to-day effect of adverse conditions on the activities, e.g. oviposition of the midges,

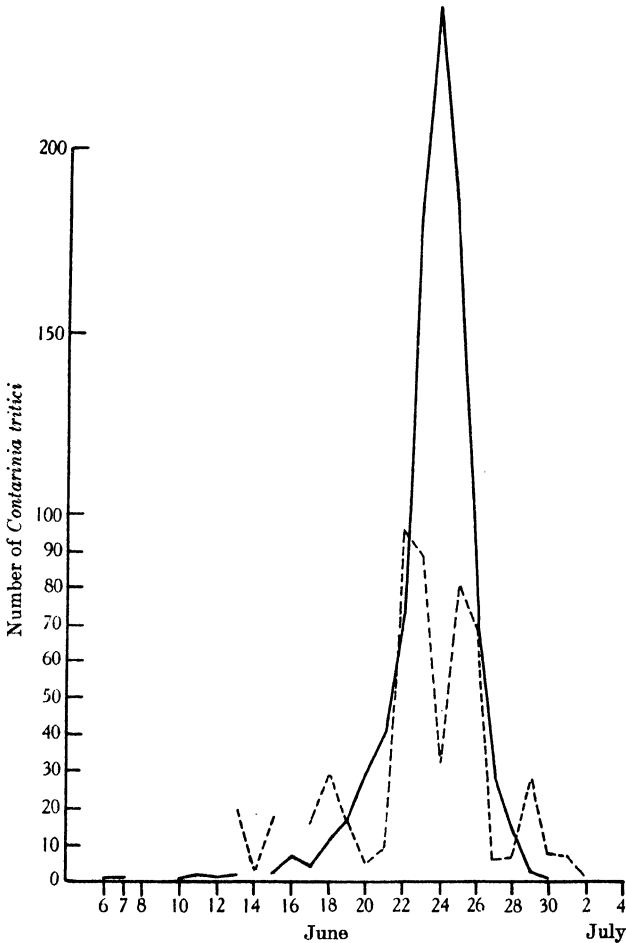


Fig. 1. Daily numbers of *Contarinia tritici*, 1935. - - - captured, — reared.

and this is probably a major factor in the determination of the subsequent population of the larvae. Breeding, under the conditions used, does, however, afford an accurate idea of the time of emergence in the field.

The sex ratios of the two species as obtained from the breeding in the outdoor insectary are set out in Table 4. The numbers reared can be ascertained by reference to Tables 1 and 2.

The numbers of *C. tritici* emerging in the August and September of the same year as the larvae have been too small to allow an estimate of the sex ratio to be made except in the summer of 1929 when 234 midges emerged. In this case the sex ratio was 50 : 50. Taking the total number of midges emerging at this period of the year from 1930-9, when 360 midges emerged, the sex ratio is 46 : 54.

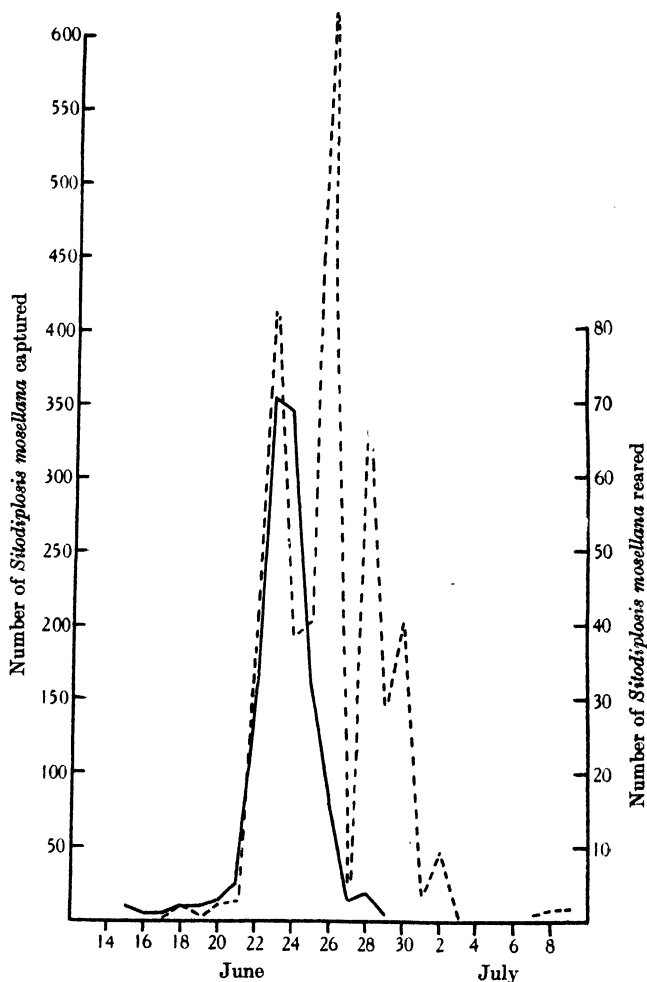


Fig. 2. Daily numbers of *Sitodiplosis mosellana*, 1935. - - - captured, — reared.

This late summer partial emergence of *C. tritici* has occurred in eleven out of the thirteen summers under observation. The dates of this emergence have been as follows: 1929, 22 August-3 September; 1930, 19-27 August; 1931, 13 August-20 September; 1932, 16-21 August; 1933, 4-8 August; 1934, 6-23 August; 1935, 18-31 August; 1936, 25 August; 1937, 10-21 August; 1939, c. 20 August; 1940, 23 August *sqq.*

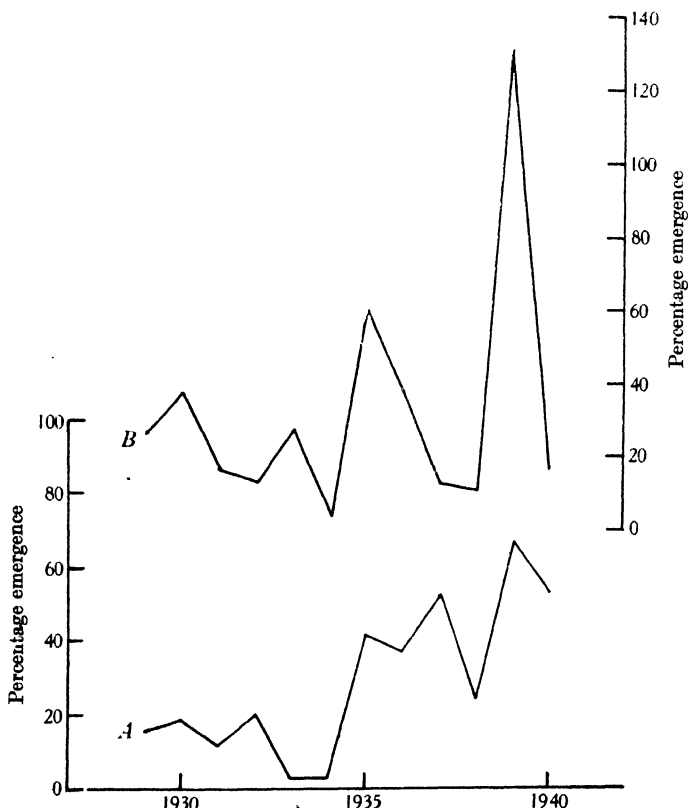
Table 3. Comparison of numbers of midges obtained by three different methods

Method	Numbers caught	Sex ratio	Larvae in 500 ears in same year	The previous year
<i>C. tritici</i>				
Handpicking, 1927	1243	0 : 100	1780	—
Mechanical trap, 1935	550	6 : 94]	4297	3362
Breeding, 1935	915	42 : 58]		
<i>S. mosellana</i>				
Handpicking, 1927	1007	0 : 100	715	—
Mechanical trap, 1935	2473	1 : 99]	4226	572
Breeding, 1935	244	36 : 64]		

Table 4. Sex ratio of *Contarinia tritici* and *Sitodiplosis mosellana*

Year	<i>C. tritici</i>	<i>S. mosellana</i>	Year	<i>C. tritici</i>	<i>S. mosellana</i>
1928-9	36 : 64	57 : 43	1934-5	42 : 58	36 : 64
1929-30	47 : 53	40 : 60	1935-6	42 : 58	41 : 59
1930-1	41 : 59	48 : 52	1936-7	39 : 61	39 : 61
1931-2	46 : 54	35 : 65	1937-8	32 : 68	33 : 67
1932-3	39 : 61	46 : 54	1938-9	45 : 55	40 : 60
*1933-4	36 : 64	45 : 55	1939-40	41 : 59	35 : 65

* The figures for this year were obtained from the extra material used, as so few midges emerged from the material obtained from Broadbalk.

Fig. 3. Percentage emergence in insectary of *Contarinia tritici* (A) and *Sitodiplosis mosellana* (B), 1929-40.

The percentage emergence of the midges from the larvae obtained the previous year has varied tremendously, from 3 to 66 % in the case of *C. tritici* and from 4 and 60 % in the case of *S. mosellana*. Of the latter species more midges emerged in 1939 than larvae put in the breeding pots during 1938, giving a figure of 131 % emergence. This phenomenon is dealt with in the next section. Fig. 3 shows the percentage emergence of the two species from 1929 to 1940. It will be seen that there is a correlation, but in four years the species did not behave in the same manner. As the conditions under which the larvae are kept over winter are constant, it would appear that the midges do not respond in the same manner every winter. It is safe to presume that the insectary conditions do give a constant relative indication of what is occurring in the field, e.g. winter mortality, since the same percentage is not always reared.

The effect of the winter on the actual survival of the midges must be an important factor in determining the subsequent potential infestation of the wheat. It has already been indicated earlier in the section that the weather conditions at the time of emergence is another important factor governing the amount of activity and so oviposition.

(b) *Larvae overwintering more than one winter*

Another factor determining the infestation of the wheat in the subsequent year by *C. tritici* is the percentage of the larvae that emerges the same year as the larvae, i.e. in August and September (see Table 1). This would not apply to infestations of *S. mosellana*, as there is no secondary or partial emergence in this species. The midges of this partial emergence of *C. tritici* breed on couch grass (*Triticum repens*), and so if a considerable number emerge in August a considerable additional population can be built up ready for the next year. For example, in the summer of 1929, 234 individuals of *C. tritici* emerged in August and, although the sample of larvae was heavily parasitized, as shown by the number that emerged in 1930, only one parasite developed sufficiently fast to emerge in September 1929. In this way the midge could get ahead of its parasites.

Yet another factor in determining infestation is the percentage of the previous year's larvae that develop into midges after spending one winter in the soil apart from actual winter mortality. Evidence has been obtained that *C. tritici* can spend two winters in the soil as larvae. One female *C. tritici* emerged in 1940 in breeding cages that had had no larvae put in since the summer of 1938.

In the case of *S. mosellana* this lengthening of the larval period appears to occur more frequently. In 1939 more midges emerged than larvae put in the pots the previous year (Table 2 and Fig. 3). This is explained by the fact that for economy the same fibre that had been used in 1937-8 for breeding the midges was used in 1938-9. That from the five *tritici* and five *mosellana* pots

was mixed and redistributed in the ten pots used in 1938, into five of which the 1938 *tritici* were placed and into the other five the 1938 *mosellana* larvae. In 1939, as already stated, 131 % *mosellana* emerged in the *mosellana* pots and 68 *mosellana* midges emerged in the *tritici* pots. (No *tritici* emerged in the *mosellana* pots.) Part of this 131 % and the 68 midges had obviously come from larvae put in during 1937. These same ten pots were kept over another winter (1939-40), no extra larvae being put in during 1939. In spite of this, 5 *mosellana* emerged in the *tritici* pots and 74 *mosellana* emerged in the *mosellana* pots. These 5 *mosellana* must have come from larvae put in during 1937, since this was the last year *mosellana* could have been put in these pots in which *tritici* larvae were placed in 1938. This is definite evidence that *mosellana* can spend three winters as larvae. Both the 68 *mosellana* that emerged in the *tritici* pots in 1939 and the 74 that emerged in the kept over *mosellana* pots in 1940 are evidence that a considerable number spend two winters as larvae in the soil. In fact, large enough numbers could easily carry over a extra winter to cause an outbreak if in one season only a small percentage of those that survived in the winter emerged, laid eggs successfully and both those larvae and the remaining rather large percentage of the previous year survived the winter and emerged the subsequent season together with a few that had been carried over two extra winters.

(c) *Numbers of larvae per infested grain*

The numbers of *C. tritici* larvae per infested grain have continued to vary considerably (cf. Table 4 (1)). Table 5 gives the average numbers of larvae per infested grain recorded from 1927 to 1940. The average numbers in the separate plot samples can be ascertained on reference to Tables 8-13 in the previous paper (1) and to Tables 10-18 in the present paper. The actual range in 1932-40 has been from 1 up to 54 in a single infested grain in 2629 occurrences. In the years 1927-31 the range was 1 to 91 in 5560 occurrences. In 1910 the number of larvae per infested grain was exceptionally low and no reason can be put forward to account for this. The sampling in 1910 was not done when most of the larvae had migrated to the ground. There seems to be no relation between the average numbers of larvae per infested grain and the dates of sampling or the total numbers of larvae found or the numbers of infested grains.

Table 5. *Average number of Contarinia tritici larvae per infested grain, 1927-40*

Year	Average number per infested grain	Year	Average number per infested grain
1927	7.8	1934	11.8
1928	10.5	1935	12.3
1929	13.3	1936	7.2
1930	13.3	1937	8.4
1931	11.4	1938	9.4
1932	7.3	1939	8.5
1933	11.7	1940	3.9

The numbers of *S. mosellana* have again been remarkably constant, the average numbers only varying from 1.1 to 1.5. The actual range in 1932-40 has been from 1 up to 12 in a single infested grain in 14,045 occurrences. In 1927-31 the range was 1 to 10 in 10,605 occurrences.

(d) *Alternative host plants*

The question of alternative host plants is an important factor in enabling extra early or late midges to survive and build up reserve populations ready to attack wheat.

Wagner (7) stated that the later summer partial emergence of *C. tritici* observed by himself and Kirby (6) oviposited on couch grass (*T. repens*). He also stated that midges emerging before the wheat ears emerged oviposit on rye. It has also been claimed that *C. tritici* can live on barley.

Similarly, it has been claimed that *S. mosellana* attacks rye, barley and oats in addition to wheat, and the present investigator (1) recorded this species ovipositing on slender foxtail grass (*Alopecurus myosuroides*).

In 1932 *C. tritici* was reared successfully to the full-grown larval stage on couch grass (*T. repens*), slender foxtail (*A. myosuroides*) and rye. Negative results with *C. tritici* were obtained in attempts to breed it on rye grass (*Lolium perenne*), meadow foxtail (*A. pratensis*) and timothy (*Phleum pratense*). Once, however, oviposition was obtained on timothy.

No experiments have been made yet with *S. mosellana*.

3. DEGREE OF INFESTATION, 1932-40

The full figures from the ten plots of percentage ear and kernel attack, number of larvae present, number of kernels attacked as well as the total number of kernels, spikelets and blind spikelets can be found in Tables 10-18.

Table 6 gives the degree of infestation by *C. tritici* and *S. mosellana* of 500 ears of wheat on Broadbalk field considered as a whole for the years 1932-40. This table is a continuation of Table 6 in the previous contribution (1). These figures will be discussed later (section 5 (b)), when the fluctuations over the whole period of fourteen years receive consideration.

Table 6. *Degree of infestation or percentage kernel attack by Contarinia tritici, and Sitodiplosis mosellana on Broadbalk, 1932-40*

	1932	1933	1934	1935	1936	1937	1938	1939	1940
<i>C. tritici</i>									
No. of larvae	7356	1474	3362	4297	708	2555	378	1116	977
No. of attacked kernels	1039	126	285	354	114	297	35	132	247
Percentage attacked	5.0	0.7	1.5	2.1	0.5	1.7	0.2	0.7	1.1
<i>S. mosellana</i>									
No. of larvae	3114	319	572	4226	2872	3420	827	1615	2291
No. of attacked kernels	2260	273	477	2988	2104	2409	676	1218	1640
Percentage attacked	10.8	1.4	2.5	17.9	9.2	13.9	3.2	9.1	7.5
No. of kernels in sample of 500 ears	20,933	18,920	18,859	16,745	22,940	17,240	20,883	17,824	22,043

4. RELATIVE PARASITISM, 1929-40

It has not yet been possible to proceed any further with the identification and biology of the parasites (1), neither have experiments yet been carried out in the field to ascertain how similar are the ratios of the emergence of the midges and parasites in the field and laboratory.

In Table 7 the total midges and parasites reared from the 1928-9 season up to date are given, as well as the relative parasitism in the various samples and the average relative parasitism for each season. In 1933-4 so few insects were reared that the figures of relative parasitism are not trustworthy.

These results are discussed in section 5 (b).

Table 7. *Relative parasitism figures, obtained by breeding in the insectary, of Contarinia tritici and Sitodiplosis mosellana, 1928-40*

Year	Total midges	Total parasites	Samples of relative or effective % parasitism					Average
			1	2	3	4	5	
			<i>C. tritici</i>					
1928-9	181	19	10	—	—	—	—	10
1929-30	2544	933	5	27	28	39	31	26
1930-1	873	979	38	69	57	54	—	54
1931-2	1928	1570	64	40	39	40	43	45
1932-3	56	152	80	77	66	66	—	72
1933-4	13	31	66	88	—	—	—	(77)
1934-5	915	432	35	20	34	30	37	31
1935-6	1378	206	8	6	12	16	19	12
1936-7	301	67	18	—	—	—	—	18
1937-8	558	42	13	2	15	3	0	7
1938-9	242	7	2.3	0	2.5	9.5	5	3.9
1939-40	588	1	0	0	0.7	0	0	0.14
<i>S. mosellana</i>								
1928-9	47	130	73	—	—	—	—	73
1929-30	125	93	40	45	—	—	—	43
1930-1	88	482	69	78	98	92	—	84
1931-2	110	614	94	90	81	80	80	85
1932-3	151	616	77	83	79	81	—	80
1933-4	8	6	37	50	—	—	—	(43)
1934-5	244	100	15	28	35	27	35	28
1935-6	1406	191	17	8	10	13	12	12
1936-7	107	255	42	91	87	81	65	73
1937-8	221	140	57	22	67	29	66	48
1938-9	1041*	43	4.2	5	5.3	4.4	1.9	4
1939-40	192	68	5	0	5.2	20	15	18

* More midges than larvae put in the previous year. For explanation see section 2 (b).

5. DISCUSSION OF RESULTS, 1927-40

(a) Emergence

As was shown previously (1) most of the *C. tritici* adults emerge during the night, though some emergence does take place throughout the day.

Full details of the emergences per week of both species are given in Tables 1 and 2 of this paper. Fig. 4 shows the actual dates of the first emergence and those of the crest of emergence of both species for each year from 1929 to 1940 inclusive. In addition, the dates of harvesting the wheat on Broadbalk are shown (1927-40) as well as the dates of 50% wheat-ear emergence from

the enveloping sheaths for the years 1933-9. These latter dates refer to the precision wheat trials on the Rothamsted Experimental Farm and not actually to Broadbalk field.

It can be seen that these six sets of data are closely correlated, especially those of the emergence of the midges and those of the 50 % ear emergence. It should be possible to predict, in the first half of June, an early or late harvest, provided one knows the dates of either midges' emergence or the 50 % ear emergence dates. Occasionally, as in 1933, the midges emerge too

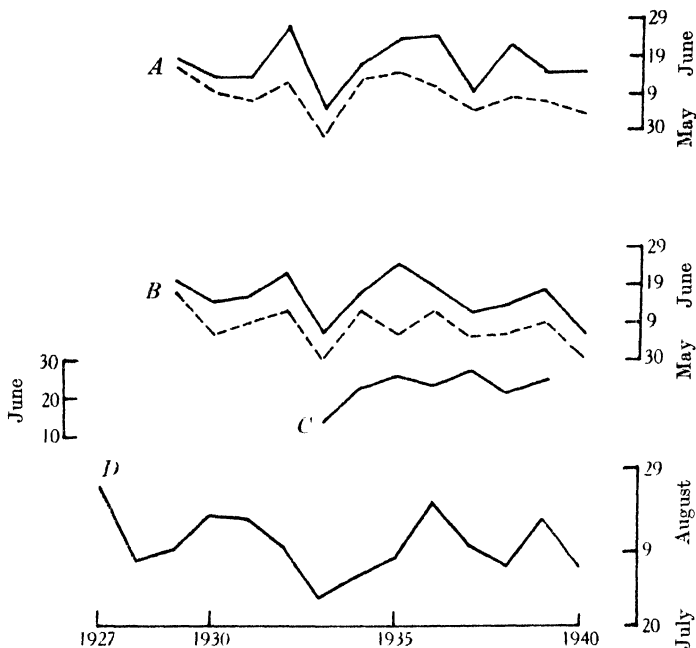


Fig. 4. Dates of first (---) and peak (—) emergence of *Sitodiplosis mosellana* (A) and *Contarinia tritici* (B), 1929-40 (C) of 50 % wheat-ear emergence, 1933-9, and (D) of harvesting Broadbalk wheat, 1927-40.

early for successful oviposition, i.e. before the wheat ears have emerged, and then they oviposit on their alternate host plants. In such a year the infestation on the wheat shows a marked decrease. For example, the infestation in 1933 on Broadbalk was 1474 larvae compared with 7356 the previous year in the case of *C. tritici*, while the corresponding figures for *S. mosellana* were 319 and 3114. This lack of coincidence of midge and ear emergence is probably a comparatively rare phenomenon. In 1940 the midge emergence was equally early, but in this case, by observation, so was the wheat-ear emergence. Consequently there was no similar decrease in numbers of midge larvae.

Both species of midge seem as a rule to reach the crest of their emergence a few days before the 50 % ear emergence date and *C. tritici* slightly before *S. mosellana*.

(b) Incidence of attack

The method of obtaining the correct date for sampling, i.e. the date when the greatest number of larvae would still be in the ears, has been to allow 3-4 weeks to lapse after the peak emergence of the midges. This latter date has been obtained by rearing the larvae of the previous year's infestation in an outdoor insectary. It has been shown (section 2 (a)) that dates of emergence so obtained approximate closely those in the field. In order to fix the exact day for sampling test examinations of the ears have been made to check the stage of growth of the larvae, and the state of the weather has also been noted. Sampling has, when possible, been done rather before rain than after, at the beginning of a week rather than just before a week-end, and so on. This is because the larvae tend to leave the wheat ears after rain and after the wheat ears have been cut. The dates of sampling, which can be ascertained from Tables 8-13 in the previous contribution (1) and from Tables 10-18 in the present paper, have varied from 8 to 16 July. They have extended from 21 to 32 days after the peak emergence of *C. tritici* and from 18 to 30 days after the peak of *S. mosellana*.

The number of larvae found in 500 ears of wheat from 1927 to 40 can be found for 1927-31 in Table 6 of the previous contribution (1) and for 1932-40 in Table 6 of the present paper. It will be seen that in the case of *C. tritici* the lowest figure has been 378 in 1938 and the highest 19,273 in 1931. *S. mosellana* was most scarce in 1933, only 319 larvae being found, and most abundant in 1931, when 6027 were present.

In Figs. 5 and 6 the numbers of larvae, the relative parasitism per cent and the percentage emergence of the midges in the insectary are depicted. The parasitism figures are plotted in the year of the midges' and parasites' emergence and not in the year in which the parasites were in the larvae. So the larvae of any one year are parasitized to the extent shown the subsequent year.

Considering *C. tritici*, it will be seen that after low numbers in 1927 and 1928 a large increase took place and high numbers were present in 1929, 1930 and 1931. Then the relative parasitism became high and the numbers of larvae fell. Subsequently the midge larvae again rose. It is thought that the low number of larvae in 1936 was purely local, perhaps even restricted to Broadbalk field. In such an event 1936 was in reality the second peak year since the study was started. An excessively heavy rainfall occurred in this year just when the peak emergence of midges was being approached, and this precipitation (slightly over the normal rainfall for the whole of June), which levelled the clay, was followed by a week of hot sunshine which in its turn consolidated the soil. No midges were observed ovipositing during this week and it is believed that no emergences could have taken place. By 1938 another trough in larval numbers had been reached. Thus in the fourteen years of the study there have been three troughs (1927, 1933 and 1938) and two peaks (1929-31 and 1935-7).

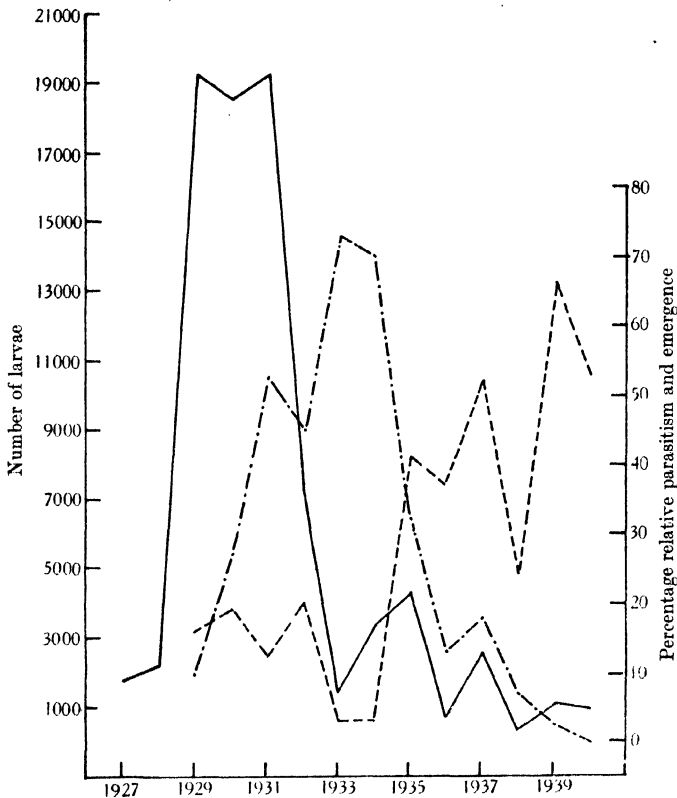


Fig. 5. Numbers of larvae (—), relative parasitism per cent (— · — ·) and percentage emergence of *Contarinia tritici* (---).

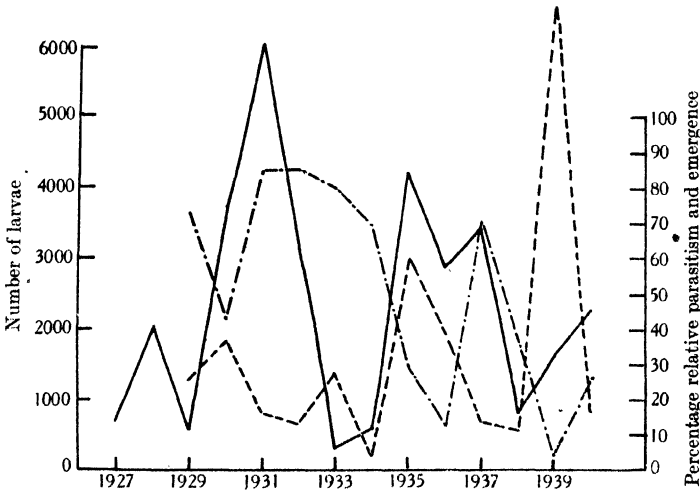


Fig. 6. Numbers of larvae (—), relative parasitism per cent (— · — ·) and percentage emergence of *Sitodiplosis mosellana* (---).

In the case of *S. mosellana* the same cycle has been encountered, two peaks (1931 and 1935-7) and three troughs (1927-9, 1933-4 and 1938). The same local disturbance in 1936 was recorded but, as would be expected owing to the slightly later date of midge emergence and oviposition, it was not so marked in this species.

The figures of relative parasitism for 1928-9 to 1939-40 have already been given in Table 7.

Referring to Figs. 5 and 6 again, it will be seen in the case of *C. tritici* that the parasitism was low in 1928-9 but then rose. Since 1935 it has been low, until in 1939-40 parasites were almost absent. The high parasitism in 1933 and 1934 might have helped in causing the reduction in numbers of larvae found in 1929-31, the first peak, but the parasitism was not sufficiently high to have caused the reduction observed in 1938-40 of the second peak (1935-7). [It has already been pointed out that the number of larvae per kernel in 1940 was unaccountably very low.] So some other factor or factors must have been responsible, just as the large fall from 1931 to 1933 can be partly accounted for by incomplete adjustment of emergence of midges and wheat ears (see previous section).

In the case of *S. mosellana* there have been two periods of high parasitism, 1931-4 and 1937, in both of which the percentage relative parasitism was high enough to reduce the numbers of midge larvae.

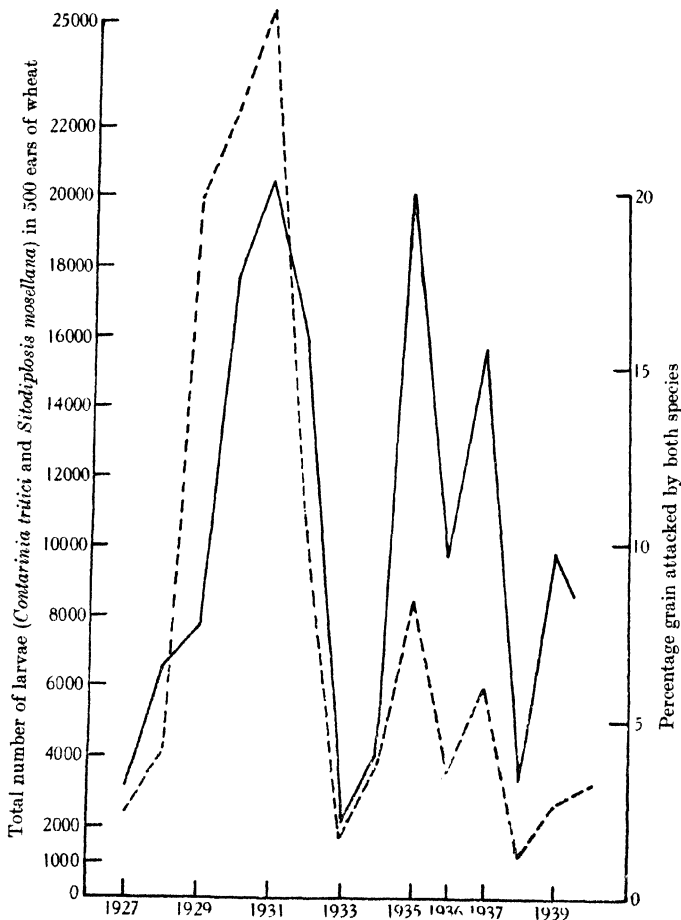
The percentage emergence is also shown in Figs. 5 and 6, because this may well affect the subsequent numbers of larvae present in the wheat.

It would appear from the graphs that years of high relative parasitism and years of low numbers are followed by years of low relative parasitism in both species of midges. It would also seem that high relative parasitism accompanies low winter survival and low relative parasitism accompanies high winter survival. This is understandable, as the parasites may easily be more hardy than the midges and, in addition, certain conditions cause the midges to postpone their emergence and spend extra winters in the soil before emerging as adult midges (see section 2 (*b*)).

It has been suggested (2, 3, 4, 5) that, considering both species together, possibly there are regular fluctuations in numbers with peaks of abundance occurring every fourth, fifth or sixth year. By observation 1916, 1920 and 1926 were peak years. In the present study nothing has disproved this suggestion. In fact it would seem that each species has a somewhat similar rhythm, as has just been shown. If, however, both species are considered together for the sake of those who do not trouble to distinguish between the two species, the rhythm does not appear to be masked in spite of the disproportionate abundance in some years of *C. tritici* and the disproportionate distribution of *S. mosellana* in the grain. For the sake of convenience the percentage grain attack and the total number of larvae are set out in Table 8 and Fig. 7. It will be seen that the data for the year 1936 upset the smoothness

Table 8. *Prevalence of the wheat blossom midges 1927-40 on Broadbalk*

Year	% grain attacked	Number of larvae	Yield in cwt./acre
1927	3.2	2,495	8.6
1928	6.5	4,238	29.0
1929	7.7	19,852	12.0
1930	17.6	22,340	15.4
1931	21.4	25,290	12.2
1932	15.8	10,470	11.2
1933	2.1	1,703	16.4
1934	4.0	3,934	22.0
1935	20.0	8,483	11.2
1936	9.7	3,580	7.7
1937	15.6	5,975	9.5
1938	3.4	1,205	24.8
1939	9.8	2,731	16.3
1940	8.6	3,268	23.5

Fig. 7. Percentage grain attacked (—) and total number of larvae (---) (*Contarinia tritici* and *Sitodiplosis mosellana*), 1927-40.

of the cycles. It has already been pointed out that the abnormal precipitation and subsequent hot week may be responsible for this. But in addition, in 1936 the Rothamsted wheat figures on Broadbalk field, regarding the effects of manurial treatments and so on, were strange as a whole although other fields on the same farm gave normal results.

In addition to the percentage grain attacked and the number of larvae, the yields of wheat are given in Table 8. It appears that when the midges are abundant the yield is low and vice versa. The correlation between the percentage of grain attacked and the yield is significant when adjustments are made to the yields 1927-31 to allow for differences in the system of cropping and fallowing (see section 7).

If some correlation between midge infestation and yield is accepted and if there is no adequate compensation in the wheat ear that has been attacked between the time of the attack and harvest, then the midges must be considered as pests. If on the other hand there is compensation, the numbers of midges can only be regarded as an indication of the size of yield, or in other words a measure of those climatic conditions during the previous months which influence the yield of wheat.¹

(c) *Factors influencing the rise and fall in population*

This study has shown definitely that there are certain periods in the year during which factors, which may be roughly termed weather factors, are of great importance in influencing the subsequent numbers of the midges and their parasites. While these factors have not been analysed, it may be useful to point out the particular periods in the year when they have their greatest effects.

For sake of convenience one can begin a survey of the year in July when the larvae are in the ears and approaching full growth. If the weather is unduly dry the fully grown larvae cannot leave the ears of wheat to pupate in the soil. Under such circumstances there is a chance that they will be removed with the crop from the field.

The next period follows immediately and extends into August. In most years a partial emergence of *C. tritici* occurs in August and sometimes early September (see section 2 (a)). Whatever the factors are which cause this emergence they can greatly influence the population the next year in either direction, increase or decrease. As this supernumerary generation can breed successfully on couch grass, it is easy to see how a very large additional increase in the population can become available to attack the wheat the following year, if this partial emergence is considerable and conditions are suitable for this midge to reach the larval stage before the winter. On the other hand, if

¹ The larvae of *C. tritici* live on the sap rising to form the grain whereas those of *S. mosellana* feed on the sap in newly formed grain causing shrunken or offal grain. It seems probable therefore that compensation in the unattacked grain is more likely to take place after an attack by *C. tritici* than after one by *S. mosellana*. If this is found to be true *C. tritici* should be considered less of an injurious pest than *S. mosellana*.

this summer emergence is considerable but its G_1 cannot develop sufficiently before the winter sets in, it may materially reduce those available for the next year's infestation.

The winter is the next critical period. It has been shown that only a small percentage of the larvae survive some winters, whereas after others a large proportion survive and emerge the following spring.

This leads on to the period, i.e. April and May, when the larvae of *C. tritici*, at least, come out of the cocoons in which they overwinter and wander about in the soil, and then pupate about eight days before emergence as adult midges. This phenomenon, together with the fact that some of the midges spend more than one winter before emerging as adults, leads one to suspect that some factors, which influence this exit from the winter cocoons and then pupation, are at work at this season of the year. If a large proportion of the overwintering larvae do not emerge as adults after one winter, an accumulation of larvae could be amassed in the soil ready to emerge when conditions are favourable.

The next critical period is at the time of emergence, i.e. June, when the wheat ears are emerging. It has been shown that when once emergence starts the peak is quickly reached and under constant conditions the emergence curve is smooth. But under field conditions several days during this emergence period are usually extremely unfavourable for activity and oviposition. A few such unfavourable days would cause a marked diminution in the number of eggs laid, especially as the adult midges normally live only 24 hr. In unfavourable weather they stay down at the base of the wheat and here the risk of being caught by spiders is considerable.

The actual reason for earliness or lateness in emergence of the adult midges is not known. It may be connected with the number of accumulated degrees of temperature through the winter or spring months. As a rule the midges emerge as the wheat ears are emerging, but occasionally these emergences do not coincide and then the midges go to their alternative host plants.

In other words, the factors influencing the earliness or lateness of the midges and the wheat are closely correlated, but there are exceptions, for example, in 1933 at Harpenden (see section 5 (a)) and in 1934 at Agassiz, British Columbia. In this latter year the height of emergence occurred on 11 June, the average date being 7 July, with the result that the earliest sowing of wheat was heavily infested and even winter wheat (late varieties) was attacked. The midges (*S. mosellana*) were a month earlier than usual, and although the wheat was earlier it was not a month earlier. In this connexion Mr R. Glendenning of the Entomological Laboratory, Agassiz, B.C., who has very kindly given the writer the above information, has written (13 Jan. 1937) that the standard indicator he uses for a safe sowing date is the blossoming of the European plum. Wheat sowings previous to this event are reasonably free from midge infestation, as they flower just before the main midge emergence.

To summarize, the following conditions would result in an outbreak of midges: (1) a large summer supernumerary emergence of *C. tritici* together

with suitable conditions for the development of the G_1 , (2) a winter of high midge survival, (3) spring conditions causing pupation and emergence following seasons during which an accumulation of larvae occurred in the soil, and (4) favourable weather at the time of emergence and oviposition.

The effect on the parasites of favourable factors for midge increase is not understood. But it has been suggested in the previous section (5 (b)) that high relative parasitism follows high numbers of larvae and low relative parasitism follows low midge numbers. This may be simply a matter of parasite food supply. But it has also been suggested that high relative parasitism accompanies low winter survival of the midges and low relative parasitism accompanies high winter survival.

6. EFFECT OF MANURING

The percentage grain infestation by the two species of midges during the years 1932-40 are shown in Tables 10-18. These are complementary to Tables 7-13, which cover the years 1927-31, in the previous contribution (1). Ten differently manured plots have been examined each year.

Plot	Treatment
2	Dung.
3	Unmanured (since 1839).
5	Complete minerals.
8	Complete minerals + 618 lb. sulphate of ammonia (S./A.).
10	412 lb. S./A. only.
11	412 lb. S./A. + $3\frac{1}{2}$ cwt. super.
12	" " + 366 lb. S./soda.
13	" " + 200 lb. S./potash.
14	" " + 280 lb. S./magnesia.
16	Complete minerals + 550 lb. nitrate soda.

No constant significant effect of manuring on the incidence of attack by either of the midges can be detected.

7. EFFECT OF FALLOWING

In 1925-6 fallowing was started on Broadbalk and for two seasons 1925-6 and 1926-7 the western or upper three-fifths of the field were fallow. In the next two seasons the lower or eastern two-fifths were fallowed. In the season 1929-30 the whole field was under wheat in five sections. In the season 1930-1 a regular cycle of fallowing was started, one-fifth of the field being fallow each year for one year. By the 1934-5 season the cycle had got into its stride, so that by sampling the four-fifths under wheat one could obtain the first, second, third and fourth crops of wheat each year after one year's fallow. Thus in five years, each fifth would have borne these crops and one cycle was completed.

It was considered advantageous to sample the field from 1935 onwards in fourths corresponding to this system of fallowing, in order to discover whether or not there was any effect on the infestation by the wheat midges. In order to get equal samples from each fourth, two extra ears of wheat were taken from each plot, making a total of 52 (4×13) from each of the ten plots sampled. The last two ears examined were discarded for the purpose of considering the infestation of 500 ears of wheat from Broadbalk as a whole.

In Table 9 the numbers of grains infested by *C. tritici* and *S. mosellana*, as well as the total number of grains in the first, second, third and fourth crops of wheat after one year's fallow, are set out for the six years 1935-40 inclusive.

Table 9. *Number of grains infested by Contarinia tritici and Sitoplosis mosellana and total grain in first, second, third and fourth crops after one year's fallow. (Sample 13 ears 10 plots \times 4 crops = 520 ears per year)*

Year	Number of kernels infested by <i>C. tritici</i>				Number of kernels infested by <i>S. mosellana</i>				Total number of grain			
	1st crop	2nd	3rd	4th	1st crop	2nd	3rd	4th	1st crop	2nd	3rd	4th
1935	68	89	141	64	733	603	883	846	4915	4256	4293	3948
1936	33	30	25	32	463	533	657	521	6750	5847	5401	5795
1937	43	78	110	78	690	486	648	702	4629	4617	4292	4385
1938	1	10	8	16	75	255	129	242	5507	5478	5261	5441
1939	18	43	31	42	257	275	360	370	5025	4470	4621	4513
1940	64	62	79	57	378	356	395	539	6294	5575	5450	5615
Total (6 years)	227	312	394	289	2596	2508	3072	3220	33,120	30,243	29,318	29,697

The lowest infestation by *C. tritici* occurred in the first crop after fallow, and the infestation of the first two crops after fallow taken together is apparently lower than the infestation of the third and fourth crops after fallow. Similarly, in the case of *S. mosellana* the first two crops are less attacked than the next two. The analysis of the infestation by *S. mosellana* in one complete cycle of fallowing, 1935-9, shows that the infestation is significantly lower in the first two years after fallow than in the third and fourth years. If the figures for percentage grain attack are used instead of the absolute numbers, these differences are shown up more. The total (for six years) percentage grain attacked by *C. tritici* is 0.69 in the first crop after fallow, 1.03 in the second, 1.34 in the third, and 0.97 in the fourth. For *S. mosellana* the figures are 8.12, 8.3, 10.5 and 10.9. If allowance is made for the positional differences in infestation in the field (the top or western fifth of the field always is comparatively more heavily infested than the rest of the field), the infestation is seen to increase, though by the third year the effect of the fallow seems to have disappeared, so that in the fourth year the infestation is no heavier than in the third year.

Thus fallowing does reduce the infestation and continuous cropping does increase the infestation by both midges.

It is interesting to note that the number of grains in the first crop after fallow is considerably greater than either in the second, third or fourth crop after fallow, but in these last three crops the grain number is about the same.

The figures for the five-year cycle agree closely with the average yields of the ten plots, viz.:

	1st year after fallow	2nd year	3rd year	4th year
Cwt./acre	17.1	14.3	12.6	12.4
No. of grains	26,826	24,668	23,868	24,082

Table 10. *Broadbalk wheat, 1932. Midge infestation on 15 July*

Plot	% ear attack		Total spikelets in 50 ears	No. of kernels in 50 ears	No. of kernels infested		% kernel attack		Total larvae in 50 ears		Av. no. larvae per kernel		Total blind spikelets in 50 ears
	T	M			T	M	T	M	T	M	T	M	
2	88	98	1147	2395	146	265	6.1	11.1	943	365	6.5	1.4	147
3	54	66	1071	1597	51	115	3.1	7.2	489	158	9.6	1.4	254
5	72	70	1082	1657	82	103	4.9	6.2	625	150	7.6	1.5	238
8	88	100	1205	2393	127	273	5.3	11.4	1099	391	8.7	1.4	195
10	62	88	1174	2109	80	199	3.8	9.4	730	268	9.1	1.3	233
11	80	96	1151	2111	114	203	5.4	9.6	835	279	7.3	1.4	219
12	84	94	1155	2127	125	248	5.9	11.2	784	363	6.3	1.5	203
13	74	96	1182	2140	99	250	4.6	11.7	570	349	5.8	1.4	214
14	82	92	1161	2164	123	263	5.7	12.2	721	346	5.9	1.3	211
16	74	96	1173	2240	92	341	4.1	15.2	560	445	6.1	1.3	200

Table 11. *Broadbalk wheat, 1933. Midge infestation on 5 July*

Plot	% ear attack		Total spikelets in 50 ears	No. of kernels in 50 ears	No. of kernels infested		% kernel attack		Total larvae in 50 ears		Av. no. larvae per kernel		Total blind spikelets in 50 ears
	T	M			T	M	T	M	T	M	T	M	
2	26	38	1110	1889	24	29	1.3	1.5	264	37	11.0	1.3	187
3	16	28	925	1408	11	24	0.8	1.7	114	31	10.4	1.3	202
5	12	30	967	1544	6	24	0.4	1.5	89	26	14.8	1.1	179
8	12	48	1111	2129	7	34	0.3	1.6	59	39	8.4	1.1	166
10	18	26	1087	2165	18	28	0.8	1.3	245	30	13.8	1.1	140
11	18	34	1033	1833	15	19	0.8	1.0	159	21	10.6	1.1	187
12	18	34	1068	2025	13	26	0.6	1.3	173	31	13.3	1.2	154
13	22	36	1048	1712	12	28	0.7	1.6	115	32	9.6	1.1	218
14	18	34	1065	1989	10	28	0.5	1.4	115	32	11.5	1.1	160
16	20	46	1107	2226	10	33	0.4	1.5	141	40	14.1	1.2	129

Table 12. *Broadbalk wheat*, 1934. *Midge infestation on 9 July*

Plot	% ear attack		% ear attack by both midges in 50 ears	Total spikelets in 50 ears	No. of kernels in 50 ears	No. of kernels infested		% kernel attack		Total larvae in 50 ears		Av. no. larvae per kernel		Total blind spikelets in 50 ears
	T	M				T	M	T	M	T	M			
2	36	46	60	1141	1929	29	41	1.5	2.1	333	52	11.5	1.3	194
3	26	50	60	1001	1512	23	47	1.5	3.1	337	51	14.7	1.1	223
5	36	44	64	1031	1345	27	42	1.7	2.7	273	53	10.1	1.3	237
8	26	50	64	1160	2098	27	45	1.3	2.1	294	60	10.9	1.3	192
10	34	54	70	1096	2262	29	66	1.2	2.9	317	76	10.9	1.2	145
11	38	60	74	1090	1834	38	61	2.1	3.3	488	69	12.8	1.1	195
12	38	50	68	1128	1910	33	33	1.7	1.7	390	38	11.8	1.2	199
13	30	52	68	1141	1850	29	39	1.6	2.1	371	41	12.8	1.1	215
14	28	56	68	1122	1868	27	56	1.4	3.0	303	73	11.2	1.3	210
16	28	42	58	1150	2051	23	47	1.1	2.3	256	59	11.1	1.3	197

Table 13. *Broadbalk wheat*, 1935. *Midge infestation on 15 July*

Plot	% ear attack		% ear attack by both midges in 50 ears	Total spikelets in 50 ears	No. of kernels in 50 ears	% kernel attack		Total larvae in 50 ears		Av. no. larvae per kernel		No. of blind spikelets in 50 ears
	T	M				T	M	T	M	T	M	
2	40	92	96	1146	37	339	1.6	15	456	12.3	1.4	176
3	26	94	94	964	14	265	1.1	21	182	13.0	1.4	281
5	20	96	96	978	14	304	1.0	22	196	14.0	1.4	270
8	40	94	94	1167	32	278	1.8	16	344	10.8	1.2	281
10	50	98	100	1112	40	323	2.3	19	501	12.5	1.5	257
11	44	88	92	1112	42	221	2.7	14	481	11.5	1.3	286
12	40	80	84	1160	35	262	2.2	16	446	12.7	1.5	294
13	36	92	92	1167	43	305	2.5	17	573	13.3	1.5	283
14	46	94	96	1098	52	265	3.3	17	630	12.1	1.3	307
16	44	98	100	1148	45	426	2.4	22	488	10.8	1.4	241

Table 14. *Broadbalk wheat, 1936. Midge infestation on 16 July*

Plot	% ear attack		Total spikelets in 50 ears	No. of kernels	% kernel attack		Total larvae in 50 ears		Av. no. larvae per kernel		Total blind spikelets in 50 ears
	T	M			T	M	T	M	T	M	
2	12	88	92	1076	9	208	0.3	8.0	4.0	1.3	96
3	12	56	60	918	9	86	0.4	4.5	7.5	1.3	104
5	14	70	74	953	9	113	0.4	5.3	7.3	1.4	96
8	14	98	98	1014	10	272	0.3	10.3	6.1	1.4	62
10	14	88	88	926	7	197	0.3	8.6	7.9	1.4	55
11	24	86	86	932	14	192	0.7	9.1	7.0	1.4	78
12	26	88	92	934	15	205	0.7	9.4	6.5	1.4	76
13	16	96	96	981	8	285	0.3	11.5	11.0	1.3	82
14	30	86	90	938	18	219	0.8	9.7	4.8	1.3	69
16	20	100	100	988	15	327	0.6	13.3	3.9	1.4	42

Table 15. *Broadbalk wheat, 1937. Midge infestation on 11 July*

Plot	% ear attack		Total spikelets in 50 ears	No. of kernels	% kernel attack		Total larvae in 50 ears		Av. no. larvae per kernel		Total blind spikelets in 50 ears
	T	M			T	M	T	M	T	M	
2	36	98	98	1085	43	267	2.0	13	8.0	1.4	190
3	42	82	84	1006	37	139	2.4	9	11.6	1.3	218
5	36	94	96	1010	24	225	1.5	14	9.4	1.5	224
8	44	94	96	1151	36	234	1.9	13	10.9	1.5	277
10	50	100	100	1101	46	322	2.3	16	7.8	1.5	200
11	26	100	100	1086	24	248	1.5	15	5.7	1.5	263
12	32	96	98	1101	23	240	1.4	15	8.0	1.5	288
13	28	94	94	1091	21	252	1.3	16	6.5	1.5	294
14	32	94	98	1093	23	231	1.4	14	7.5	1.3	288
16	24	98	98	1074	20	251	1.2	15	8.7	1.3	243

Table 16. *Broadbalk wheat*, 1938. *Midge infestation on 11 July*

Plot	% ear attack		Total spikelets in 50 ears	No. of kernels	% kernel attack		Total larvae in 50 ears		Av. no. larvae per kernel		Total blind spikelets in 50 ears
	T	M			T	M	T	M	T	M	
2	8	34	1117	2041	4	33	45	44	11.3	1.3	164
3	16	36	994	1869	11	50	122	60	11.1	1.2	145
5	8	42	1043	2018	5	68	61	85	12.2	1.3	147
8	2	26	1077	2100	1	29	20	41	20.0	1.4	139
10	4	70	1014	2150	2	105	21	121	10.5	1.2	111
11	8	54	1035	2035	4	84	50	104	12.5	1.2	118
12	6	50	1072	2132	3	69	16	77	5.3	1.1	133
13	0	56	1065	1995	0	76	0	96	—	1.3	160
14	2	58	1051	2188	1	78	1	90	1.0	1.2	119
16	6	54	1072	2335	4	84	42	109	10.5	1.3	116

Table 17. *Broadbalk wheat*, 1939. *Midge infestation on 14 July*

Plot	% ear attack		Total spikelets in 50 ears	No. of kernels	% kernel attack		Total larvae in 50 ears		Av. no. larvae per kernel		Total blind spikelets in 50 ears
	T	M			T	M	T	M	T	M	
2	22	76	1132	2189	16	107	86	131	5.4	1.2	165
3	8	58	958	1500	5	65	58	90	11.6	1.4	188
5	26	70	995	1622	18	91	143	117	7.9	1.3	204
8	12	76	1201	1780	6	106	43	123	7.1	1.2	255
10	26	86	1086	1742	17	154	166	204	9.8	1.3	212
11	26	88	1086	1827	13	168	95	241	7.3	1.4	204
12	24	76	1103	1819	18	150	184	197	10.2	1.3	211
13	16	88	1077	1399	10	130	77	169	7.7	1.3	249
14	16	82	1090	1690	14	141	150	210	10.7	1.5	214
16	20	82	1123	2056	15	106	114	133	7.6	1.3	180

Table 18. *Broadbalk wheat*, 1940. *Midge infestation on 8 July*

Plot	% ear attack		Total spikelets in 50 ears	No. of kernels in 50 ears	No. of kernels infested		% kernel attack		Total larvae in 50 ears		Av. no. larvae per kernel		Total blind spikelets in 50 ears
	T	M			T	M	T	M	T	M	T	M	
2	42	88	1088	2117	37	168	1.7	7.9	132	258	2.8	1.5	139
3	28	78	974	2057	21	138	1.0	6.7	63	176	3.0	1.3	119
5	22	86	982	1948	16	156	0.8	8.0	83	215	5.2	1.4	132
8	38	84	1139	2299	23	166	1.0	7.7	55	234	2.0	1.4	133
10	32	88	1039	2624	24	208	0.9	7.9	134	280	5.6	1.3	75
11	34	86	1064	2230	26	145	1.2	6.5	179	184	6.9	1.3	122
12	40	84	1093	2255	28	146	1.2	6.5	78	208	2.8	1.4	123
13	38	84	1109	2093	22	142	1.1	6.8	93	200	4.2	1.4	147
14	38	92	1076	2102	25	177	1.2	8.4	82	259	3.3	1.5	144
16	36	90	1104	2318	25	194	1.1	8.4	78	277	3.1	1.4	124

In other words, the increase in yield after fallowing would appear to be the result largely of an increase in the number of grains per ear; the other two factors which control the yield, number of ears per acre and weight per grain are apparently not affected by fallowing. In addition the number of grains is not affected by manurial treatment, although the weight per grain is.

8. RELATION OF NUMBER OF BLIND SPIKELETS TO YIELD OF WHEAT

There is a strong negative correlation between the number of blind spikelets and the number of grains at the time of sampling. Besides this, there is a negative correlation between the number of blind spikelets and the total possible number of grains. In Fig. 8 the dotted line represents the number of blind spikelets per year arranged in order of the fewest to the most.

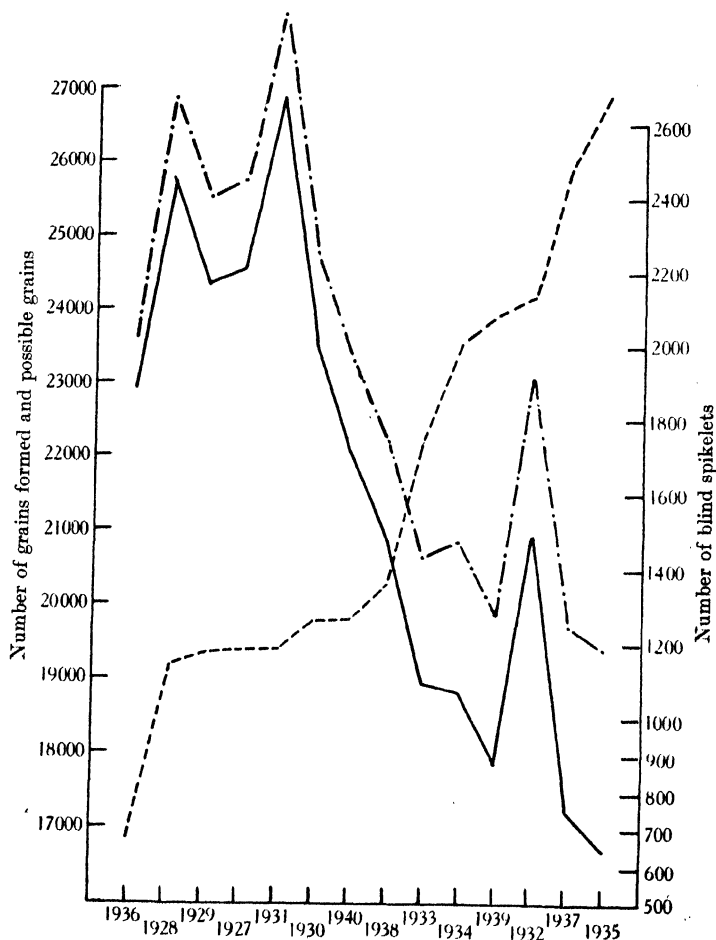


Fig. 8. Number of blind spikelets (---), number of grains formed (—) and the number of possible grains (---), arranged in order of years of fewest blind spikelets to those of most.

The years are indicated for sake of convenience. The full line represents the number of grains that actually formed, while the line-dot-line is the number of possible grains that could be formed. It is seen that in years when there is a high number of blind spikelets, besides being a low number of grains actually formed, there is also a small number of possible grains.

9. SUMMARY

1. This is the continuation of the investigation started in 1927 of the wheat blossom midges, *Contarinia tritici* Kirby and *Sitodiplosis mosellana* Géhin, as they occur on the field of permanent wheat (Broadbalk) at Rothamsted Experimental Station.

2. Data are given for the years 1932-40 relating to their biology, viz. emergence, number of larvae per infested grain, larvae overwintering more than one winter and alternative host plants, as well as to the degree of infestation of the wheat and the relative parasitism of the midges.

3. The results of the whole fourteen-year continuous study are considered.

4. The date of emergence of the midges is correlated with the wheat-ear emergence, and it appears that both are correlated with the harvesting dates. Thus knowing the dates of either the midge's emergence or the wheat-ear emergence, one can apparently predict in June the date of harvest.

5. There seem to be cycles (about five years) of abundance of *C. tritici* and *S. mosellana*, either considered separately or together.

6. Years in which there are high numbers of wheat midge larvae are followed by years of high relative parasitism and years of low larval number are followed by years of low relative parasitism in both species of midge.

7. High relative parasitism accompanies low winter survival of midges and parasites, low relative parasitism accompanies high winter survival.

8. The percentage grain attack is negatively correlated with the yield of wheat. If there is no adequate compensation in the wheat ear that has been attacked between the time of the attack and harvest, then the midges must be considered as pests. If on the other hand there is compensation, the numbers of midge larvae can only be regarded as an indication of the size of yield or in other words a measure of those climatic conditions during the previous months which influence the yield of wheat.

9. Manuring does not affect the intensity of midge attack.

10. The effect of one year's fallowing is to reduce the infestation by the midges, although this is somewhat masked on Broadbalk field by positional differences in infestation. One part of the field is always comparatively more heavily infested than the rest. This effect of fallowing seems to have disappeared by the third successive crop, i.e. non-rotation increases the infestation by both midges.

11. The effect of one year's fallowing on the number of grains of wheat is a marked increase in the first crop after the fallow. The number of grains in the second crop after fallow, while considerably less, is appreciably greater than that of the third and fourth crops.

12. In the years when the number of blind spikelets of wheat is high, the number of possible grains and the actual number of grains formed are low. Thus by estimating the number of blind spikelets between 5 and 16 July one can obtain an early estimate of the number of grains per ear at harvest. But unfortunately the number of grains formed per ear does not necessarily indicate the yield, since the yield would be measured by the number of ears or grains per acre and the weight per grain.

10. ACKNOWLEDGEMENT

I am very much indebted to Mr J. W. Weil of the Statistical Department for the great help I have derived from discussing various points with him and for his aid in expressing some of the results.

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THE TEMPERATURE PREFERENDUM OF CERTAIN INSECTS¹

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(With Plate 9 and 10 Figures in the Text)

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1. INTRODUCTION

ALTHOUGH much work had been done on the optimum temperature for various insects, and the maximum and minimum temperature necessary to maintain life, relatively little has been done on their preferred temperature. In fact, at the time the present work was started only one worker in England had ever published on this subject.

In order to avoid any possible confusion between optimum and preferred temperatures it might be well to define them before going further. Although it is realized there are many definitions of the optimum temperature of an insect, for the purpose of this work it is taken to mean the temperature at which the greatest number of insects are reproduced in a given period of time. In other words, it is the temperature at which the relation between the number of eggs laid, the length of life and the mortality is such that the largest numbers of individuals survive after a given time. The temperature preferendum, on the other hand, is the temperature to which an insect moves if given its choice of a temperature gradient.

¹ Part of a Thesis presented to the University of London for the Degree of Ph.D.

The term 'preferendum' was proposed by Dr C. B. Williams while in Egypt, in 1922, although he never published on this subject himself. Since then various workers have adopted the term in one form or another. Herter has used 'thermische Indifferenzzone' and 'thermotaktisches Optimum'. Bodenheimer & Schenkin used 'Vorzugstemperatur'. More recently Gunn has suggested the term 'eccritic' temperature.

Temperature preferendum work may be approached from three different angles. (a) A comparative study of the temperature reactions of a large number of representative insects from various orders. (b) A comparative study of such factors influencing the temperature reactions as: age (larvae and adults), sex, insects tested with and without food, insects previously kept at room and at a constant high temperature, and the environment or natural habitat of the insect (those inhabiting soil, water, stored products, and also parasitic insects, etc.). (c) A detailed physiological study of one species to determine why it prefers a certain temperature or why it reacts as it does. Obviously this latter work could not be undertaken until (a) and (b) had been at least partially investigated. Because of a time limit, as much of the present work as possible was done under the first two groups.

The writer wishes to thank in particular Dr C. B. Williams for suggesting this problem and for his always unfailing help and advice; also the other members of the Entomology Department of Rothamsted Experimental Station for the helpful atmosphere found there.

2. PREVIOUS LITERATURE

As stated above, the first to carry out work on the temperature preference of insects was Williams in 1922 in Egypt. However, this early work was never published and the only reference to it is Kirkpatrick (1923) in his work on the Egyptian cotton-seed bug (*Oxyacrenus hyalinipennis*). Kirkpatrick states: 'The resting bugs were found, by means of an apparatus devised by Mr C. B. Williams, to be thermotropic to a temperature of 36° C.'

Herter (1923 *a* and *b*, 1924, 1925) carried out some preferendum studies on insects. His apparatus ('Temperaturorgel') consisted of a cage 61 cm. long, 10 cm. high and 3 cm. wide, with an aluminium floor, a cardboard roof and glass sides. The metal floor was heated at one end with a gas flame and the heat lost to the opposite end caused a gradient to be set up. Thermometers were projected through the cardboard roof. The difficulty with this apparatus was that, owing to the lack of insulation, the temperature gradient within the cage varied with the room temperature. In his work with wood-ants (*Formica rufa*) Herter showed that the temperature of the environment from which the insect was taken influenced its preferred temperature. Figures were quoted which suggested that with an increase in the initial temperature there was a corresponding increase in the preferendum, i.e. that the preferendum varied directly with the temperature of the insect's previous habitat.

Parker (1924), in his work on the clear-winged grasshopper (*Camnula pellucida*), placed fifty first and second instar nymphs on a covered glass plate which was heated in the centre by an electric lamp from below. The temperature ranged from 60° C. above the lamp to 18° C. at the edge of the glass. The grasshoppers arranged themselves in a definite circle around the central area at a temperature of 37-38° C. The experiment was repeated several times. In connexion with an insect's preference for *radiant heat* mention should be made of the early work of Lodge (1918). In working on the sense reactions of house-flies it was observed that they congregated in large numbers round a lighted bunsen-burner and arranged themselves in a very definite circle, the size of which varied according to the distribution of heat. The temperature of this circle was 42-44° C. Heat distribution was controlled by placing larger or smaller pieces of asbestos over the flame, when the flies arranged themselves in larger or smaller circles respectively. If the gas was turned off the flies ceased to sit in a ring, but came closer to the base of the burner.

Kirkpatrick (1925), working with mosquitoes (*Culex pipiens*), provided them with water in troughs on top of a bar of aluminium heated at one end. The water varied from 14 to 36° C. and the females showed a decided preference for water at temperatures of 21-23° C. for oviposition.

Bodenheimer & Schenkin (1928) repeated Herter's early experiments with several insects. They found that the flour weevil (*Tribolium confusum*) had a preferendum of 24-760 26-568° C. if previously kept at a temperature of 15-18° C. However, beetles kept for a month in a constant temperature of 25° C. preferred a zone between 9-118 and 10-740° C. They also found that the average preferred temperature of nymphs of the house cricket (*Gryllus domesticus*) was 20-40° C. in Palestine; while Herter gives 26-512° C. to be its preferendum for Germany. As will be noticed the temperature readings were made to the third decimal place; this seems unjustified since, in most cases, the insect's preferred zone covered several degrees.

Fulton (1928), working independently, devised a preferred temperature apparatus based on an entirely different principle. A trough filled with wet sand was packed in ice at one end, while the opposite end was heated by electric lamps. The wet sand conducted the heat sufficiently well to form a temperature gradient although the changes were greater near the ends. Insects were placed in a glass tube which was imbedded in the sand leaving only the top exposed. Thermometers were pushed into the sand in a slanting direction so that the bulbs were under and in contact with the glass tube. Using adult click beetles (*Melanotus communis*), Fulton found that 57 % came to rest within the range of 26-29° C. The larvae showed no marked preference for any temperature between 17 and 29° C.

Bodenheimer's (1929) work on the desert locust (*Schistocera gregaria*) comprised all stages of the insect. In the development of the young there was a progressive increase in preferred temperature from 29-4° C. for the first stage

nymphs to 36.7° C. for the fifth stage nymphs. Bodenheimer concludes that this rise in the preferred temperature is connected with physiological changes in the insect during development, and also suggests that it is possible that this is connected with the water contents of the insect's body.

Grossman (1929) describes the results of his experiments with the Mexican cotton boll weevil (*Anthonomus grandis*). For this work he employed an apparatus which consisted of a 3 ft. length of $\frac{1}{2}$ in. glass tubing placed within a slightly shorter tube 2 in. in diameter. The space between the smaller and larger tubes was filled with water and sealed off with a cork at the ends. By heating the water at one end of the apparatus and chilling it at the other, a temperature gradient extending from 9 to 60° C. was obtained in the inner tube. In these experiments the weevils only reacted by avoiding temperatures above 49° C.

Bodenheimer & Klein (1930), working with ants, arrived at the conclusion that the preferendum of each species remains practically constant in different months regardless of the differences in the environmental temperatures. Hertzner (1930), working on the behaviour of the Argentine ant (*Iridomyrmex humilis*), used a rather crude temperature apparatus. It consisted of a galvanized pan, 3 ft. long, the bottom covered with soil, with a jar of ice at one end and an electric lamp at the other. The temperature gradient ranged from 13 to 50° C. Using worker-ants with pupae he found that the ants would settle down with their young between 21 and 27° C.

In further illustration of an insect's previous environment affecting its preferred temperature the work of Bodenheimer (1931) may be cited. He suggested that the humidity of the air in which insects are kept before an experiment has an effect on their preferred temperature. For instance, the Tenebrionid beetle, *Adesmia clothrata*, when kept in moist air before an experiment, preferred a temperature of 39.4° C., but when previously kept in dry air, preferred 36.6° C. Gunn (1931) obtained similar results using the cockroach (*Blatta orientalis*). Fahmy (1931) constructed a replica of the original temperature-preference apparatus used by Williams in Egypt. Working with *Plinus tectus* he found that they preferred a temperature varying from 22 to 25° C. with an average of 23.5° C.

Uvarov (1931) has reviewed the literature on insect preferendum. He states that temperature preferendum 'is probably one of the most potent factors influencing the ecological distribution of insects and their movements', and suggests that the preferendum should be determined for each stage of every insect of economic importance. Nieschulz (1933, 1935) gives a useful discussion on the subject of optimum and preferred temperatures. He investigated the reactions of *Stomoxys calcitrans* to various temperatures. The average optimum was found to be 27.7° C. for the female and 28.8° C. for the male. The relative humidity of the atmosphere was said to be of negligible influence. This optimum temperature was about the same as that at which the normal activity started

to increase. It was found the results were not affected by atmospheric humidity, sex, age, amount of feeding, or egg maturation. The preferred temperature of *Stomoxys calcitrans* was found to average 29.4° C. for freshly captured females and 25.9° C. for males. For females of *Culex pipiens* that had hibernated in a cellar the temperature preferences was 8.9° C.

The following is a comparison of the results obtained by Nieschulz on the reactions of *S. calcitrans* to temperature:

Optimum temperature °C.		Preferred temperature °C.	
Males	Females	Males	Females
28.8	27.7	25.9	29.4

The preferred temperature for the males is almost 3° less than their optimum temperature, while for the females it is 1.7° more than their optimum temperature. These facts are pointed out here since so few investigators of preferred temperature have ever conducted experiments on optimum temperature so that comparisons might be made. Experiments were also carried out by Nieschulz with *Musca domestica* and *Fannia canicularis* similar to those with *Stomoxys calcitrans* above. Optimum temperature for *M. domestica* showed an average of 33° C. for females and 34.2° C. for males. The optimum for both sexes of *F. canicularis* was 23.7° C. A comparison of these results with those for *S. calcitrans* shows that all three flies have typical optimum temperatures that are peculiar to the species.

Martini & Teubner (1933), working with mosquitoes, found the preferred hibernation temperatures were 6-8° C. for *Anopheles maculipennis* var. *messae* and 10-13° C. for *A. maculipennis* var. *atroparvus*. In the field the former also appeared to avoid high temperatures and high saturation deficiencies more than the latter. Here there was a considerable difference in choice of temperature even between two varieties of the same species. In 1934 Nieschulz carried out further experiments on the temperature preferred by *Stomoxys calcitrans*, the results of which confirmed those published the previous year.

The most exact study of preferred temperature is found in the work of Gunn (1934) on the cockroach. In his experiments he used a temperature-gradient apparatus in the form of a copper trough electrically heated at one end and cooled by ice at the other. The entire trough was well insulated, so that a constant gradient could be maintained irrespective of the room temperature. With this apparatus it was found that the preferred temperature range of an adult male *Blatta orientalis* was 20-29° C., and that this temperature range was not affected by changes in the air humidity.

Herter, in his later work (1934, 1936), used a modified form of his original apparatus in which he inserted thermometers into the aluminium bar, or floor of the gradient. With this apparatus he found the preferred temperature

(which he calls thermotactic optimum) for the flea, *Archaeopsylla*, to be 34.4°C ., and for *Cimex*, 35.7°C . These figures are said to agree well with the surface temperature of the insect's hosts. Hertex also experimented with small mammals. Using mice and bats it was found the smaller forms had the higher temperature preference. Results with long-eared bats indicate that the ear perhaps plays a role in temperature perception and so indirectly is a factor in determining the animal's preferred temperature or 'thermotactic optimum'. Herter also recorded the frequency with which orientation occurred with the head toward the cool or heated end of the apparatus.

Nicholson (1934), in working on the influence of temperature on sheep blow-flies, determined the preferred temperature in a different way. The number of flies on the corks of the observation jars at various temperatures was recorded, and it was observed that this number was greatest near the extremes of temperature. Because of their low conductivity the corks probably felt warmer at very low temperatures and cooler at high temperatures, thus being less uncomfortable than the rest of the jar. In these experiments the greatest number of insects gathered on the corks at temperatures below 20°C . and above 35°C . Thus the range between 20 and 35°C . was considered to be the preferred temperature.

Continuing his work on the cockroach, Gunn (1935) made a comparison of the temperature preference of three different species. He found the upper limit of preferred temperature for *Periplaneta americana* and *Blattella germanica* to be 33°C ., while for *Blatta orientalis* it was 29°C . The lower limit was not so sharply defined and it was suggested that further work be done before it could be regarded as significant.

Thomsen & Thomsen (1937, 1938) determined the preferendum of certain Dipterous larvae in their natural habitat. The temperature gradient apparatus used allowed the larvae to move freely in dung with a temperature range from 9 to 50°C . This overcame two serious weaknesses of the usual preferred temperature experiment: (a) the insects were in their natural medium and had access at all times to their food, (b) the relative humidity was fairly uniform throughout the apparatus. Larvae of *Musca domestica* in horse dung showed a definite temperature preference, which varied with their age. Larvae that were feeding preferred temperatures between 30 and 37°C ., while those ready to pupate preferred temperatures below 15°C . The preference shown by feeding larvae corresponded to that found in their natural habitat in a fermenting manure heap. Within the manure a temperature as low as 15°C . cannot be obtained, hence pupae are to be found in the earth nearby. The optimum temperature for the development of house-fly larvae was shown to be 34°C . Supplementary experiments indicated that the attraction of the larvae to a given zone of the dung layer depended not solely on its temperature, but also on a chemical change therein. This chemotaxis was particularly marked only during the second day of larval life. The authors conclude that

the vertical distribution and movements of the larvae in natural conditions depend chiefly on their temperature preference and to a lesser degree on chemotaxis, negative phototaxis, hygrotaxis and thigmotaxis.

Larvae of *Stomoxys calcitrans*, which live in litter containing cow dung at 20-30° C., had a preferendum between 23 and 26° C. Comparing the temperature preferences of the larvae of these two species with those obtained above by Nieschulz for the adults it will be seen that the same differences hold. Namely, *Musca domestica* prefers a higher temperature in both the larval and adult stages than does *S. calcitrans*.

The first attempt to determine the preferred temperature of soil insects was made by Campbell (1937) in his work on wireworms. A modified form of Fulton's temperature-preference apparatus was constructed in which wet sand was used for the conductivity of heat and cold. The preferendum for these insects was lowest (17-23° C.) in the period February and March. During the summer the preferendum rose until the high point (27° C.) was reached in September, and then dropped in the latter part of September and October. In general, the experiments indicated that the preferred temperature varies with the season, being higher in the summer and autumn than in the winter and spring. It was also shown there is a lag behind the natural soil temperature, indicating that the preferendum does not change until the wireworms have been subjected to the higher or lower temperatures for a month or more.

Thomson (1938) used an alternative chamber to determine the reactions of mosquitoes, *Culex fatigans*, to temperature and humidity. This apparatus was roughly of the same principle as that used by Gunn & Kennedy (1936) to determine the reactions of insects to humidity. The maximum difference of temperature between each side of the chamber was only 10° C. All stages of the mosquitoes showed a strong avoidance of high temperatures. This was strongest in the hungry females, less strong in the blood-fed females and those with mature ovaries, and least strong in the newly emerged females. Gunn & Cosway (1938) tested cockroaches for their preferred temperature at two different humidities—moist and dry. In general, the average preferred temperature of these insects was higher in moist air than in dry. Individual cockroaches which did not react to humidity at a constant temperature likewise ignored the humidity in a temperature gradient. However, individuals which did react to humidity at a constant temperature also reacted to it in a temperature gradient by going to a lower temperature in dry air than they did in moist.

The only work on the reactions of aquatic animals to a temperature gradient is a recent paper of Doudoroff's (1938) on fishes. An apparatus is described whereby a steep horizontal temperature gradient can be maintained without the presence of vertical gradients in the water. Several species of marine fishes, chiefly *Cirella nigricans*, showed a marked selection for temperatures which were relatively high in comparison with the normal environment of the

fishes. While acclimatization was found to influence selection, the effect was slight and only temporary, the selected temperature being, to a large extent, independent of past experience. It was concluded that selection is indicative of the relative stimulative or detrimental effects of given rapid changes of temperature, while the common view that such selection indicates the nature of 'optimal' conditions or habitat preference is not tenable.

3. DESCRIPTION OF TEMPERATURE GRADIENT APPARATUS

Since the various pieces of apparatus used in the present work are new, or modified, a brief description is necessary. It should be mentioned that all were made in the Rothamsted workshop and were relatively inexpensive. The total cost of the two most expensive items, the linear brass gradients, was under £2 each.

(a) *Williams's early apparatus*

Plate 9A is a photograph taken by Dr C. B. Williams of his original temperature-preferendum apparatus. When the present work was started, the first temperature gradient to be constructed and tried was a replica of this. It consisted of a glass tube $1\frac{1}{2}$ in. in diameter and 40 in. in length, supported at each end by a retort stand with clamps. 'Compo' tubing, $\frac{1}{4}$ in. in diameter, was tightly coiled in a spiral fashion about the glass tube and looped down at one end with a small gas flame on the steep slope. At one side both ends of the compo tubing were joined to a Y tube, which in turn was joined to a glass funnel by means of rubber joints. This funnel was for filling the coils with water. In order to prevent air locks no water was used in the coils which had not previously been boiled. Compo tubing of a low melting point, which cannot be passed directly into a flame, should be immersed in a hot water bath which in turn may be heated by a bunsen burner. For taking temperatures a thermometer, with a thread tied to each end, was inserted into the glass tube and the strings allowed to hang free at the ends. The thermometer could thus be pulled along in either direction and the temperature recorded at any position in the apparatus where insects were gathered. The ends of the glass tube were plugged with cotton wool.

By heating the water at one point and setting up a circulation through the compo coils, a gradient could be maintained from a high temperature (about 35° C.) at one end of the apparatus down to room temperature at the opposite end. Inversely, if the compo loop were cooled instead of heated a gradient could be made from a low temperature at one end up to room temperature at the other end.

The great advantage of such an apparatus was that an insect had at all times a choice of a series of gradients. Each space between two compo coils offered a small independent gradient. Above the preferred temperature the insects tend to group in the cooler space between the coils, and below the preferred temperature in the warmer area on the coils. After a few trials, this apparatus was abandoned in a search for one which offered a complete gradient from a low to a high temperature.

(b) *Brass trough with ice-box* (Pl. 9B and Fig. 1)

This apparatus was made in the form of a trough built up from solid brass strips $\frac{1}{2}$ in. in thickness. The inside depth was 1 in., the inside width 2 in., and the overall length 4 ft. The trough was blocked off $3\frac{1}{2}$ in. from each end by soldering in place a brass piece of the same thickness as the sides.

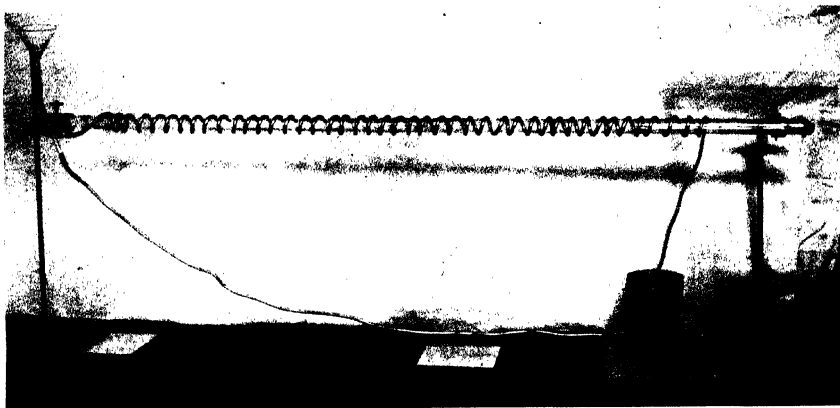


Photo A. Apparatus used by Williams in 1923.

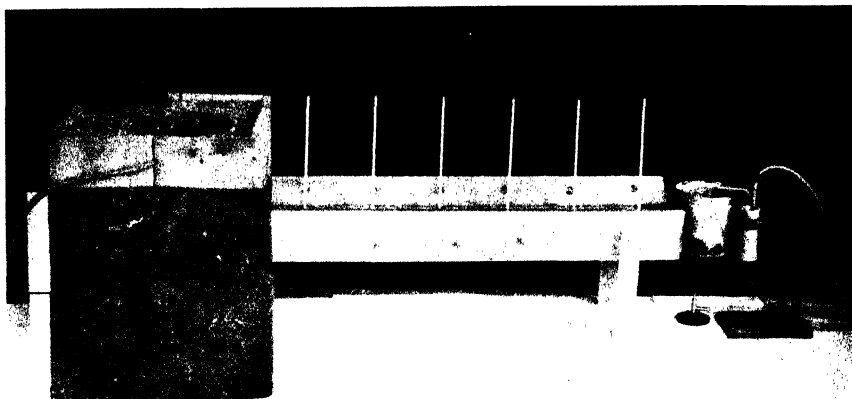


Photo B. Gradient apparatus with hot bath and ice-box. (Thermometers 6 in. apart.)

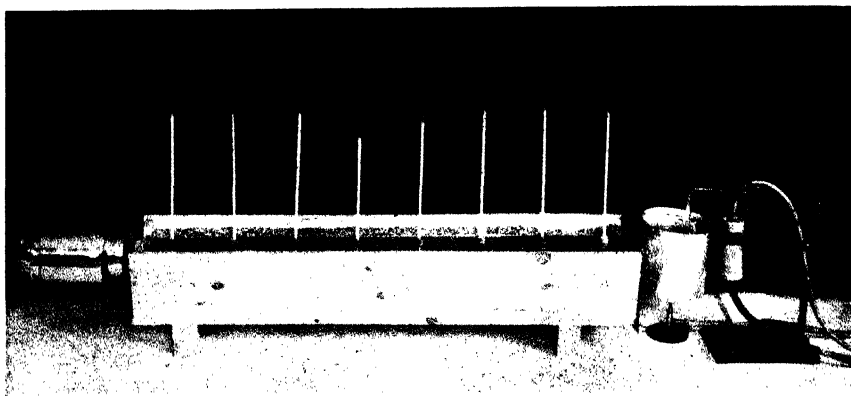


Photo C. Gradient apparatus with hot bath and Thermos jar. (Thermometers 5 in. apart.)

A circular tin 6 in. in diameter by 6 in. in depth was secured and a hole made into one side 2 in. from the bottom. The trough was projected through this opening into the tin for a distance of $3\frac{1}{2}$ in., and the tin soldered tightly about the brass end-piece. This was to serve as a hot water bath for heating the hot end of the gradient.

For the other end of the trough a larger tin was secured (a 28 lb. honey pail), 8 in. in diameter by 12 in. in depth, and with a tightly fitting cover. A hole was made in the can 2 in. from the bottom and the free end of the trough projected into the tin the same distance as for the opposite side, and soldered in a like manner. Since this honey pail was to store the ice for the cold end of the gradient good insulation was necessary. First it was wrapped with cotton wool. Then a wooden box, 18 by 18 in. and 21 in. deep, was built around it so that the top of the tin was just level with the top of the box. The box was filled with granulated cork, which was firmly packed about the tin. Near the top of the tin was fitted an overflow outlet so that the water from the melting ice could drain off. The entire box was then covered with a square of thick felt as used for covering bee hives, and over this was a wooden lid.

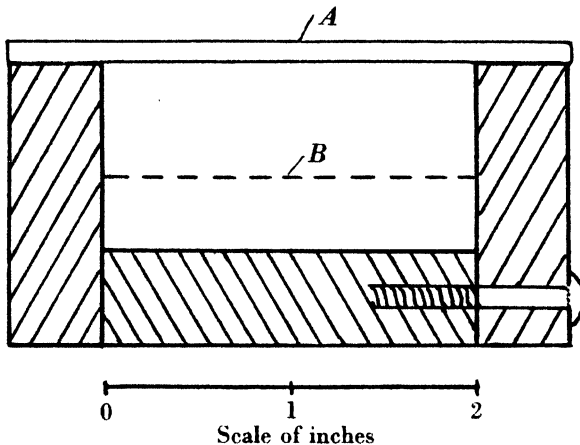


Fig. 1. Cross-section of linear apparatus. *A*—glass top. *B*—false floor of organicie.

There was thus left an actual run-way in the trough of $37\frac{1}{2}$ in. for the insects. (Notice that $3\frac{1}{2}$ in. of the gradient which ran from the ice chamber to the outside of the insulation box was lost for experimental work.) In order to prevent temperature loss from the metal gradient it was also lagged in the same way as was the ice container. First with cotton wool and then with granulated cork. To hold the cork a wooden trough 8 in. wide and 4 in. deep was built to fit about the entire exposed length of the brass channel. At the cold end this trough fitted flush with the ice-box, while at the hot end it was covered with tin-plate to protect it from the bunsen burner.

The cover to the gradient consisted of six glass plates each $6\frac{1}{4}$ in. long, 3 in. wide, and $\frac{1}{8}$ in. in thickness. The ends of each plate were ground so as to fit tightly to the next and a hole was drilled in the centre of each to admit a thermometer. The glass covers were then sealed in place with vaseline. The rubber stoppers used to hold the thermometers in position were first 'weathered' out of doors for 3 months. This was to avoid any toxic emanation from the new rubber as discussed by Mellanby & Buxton (1935). A piece of wall board $\frac{1}{2}$ in. thick, 3 in. wide, 38 in. long and with holes bored to allow for the thermometers to project through was then placed over the glass. The primary purpose of this cover was to darken completely the experimental chamber whenever this was desired. However, it was also

beneficial in serving as additional insulation. A 95 cm. rule was placed alongside the trough for determining the position of the insects.

The hot water bath was kept at a uniform level (about 1 in. from the top of the tin) by means of a constant level apparatus (Fig. 2). This was held in place by a retort stand and was supplied with a constant stream of water from a nearby tap with an overflow to the sink. The heat for the water bath was supplied from a micro bunsen burner which was fitted to burn night and day.

With this apparatus a temperature gradient over a 3-day period (the length of each experiment) averaged from about 10° C. at the cold end, when cooled with ice and salt, to 35° C. at the hot end. There was but little variation from hour to hour except at the cold end, which fluctuated between 5 and 10° C. The temperature gradient was approximately a straight line allowing the insects a choice of about 1° C. in every 4 cm.

(c) *Brass trough with Thermos vacuum flask* (Plate 9 C)

This apparatus was also of brass and was constructed in practically the same way as the above gradient. The only difference being that the ice for the cold end was contained in a 1 gallon Thermos vacuum flask instead of a lagged ice-box. One end of the trough was projected through the flask's cork for 4 in. and the cork sealed in place about the trough with marine glue. The flask could then be filled with ice and salt and slipped on and off the cork at the end of the gradient.

By using the vacuum flask, and thus eliminating the large amount of lagging, an additional 2½ in. of the trough were made available for experimental purposes. This gave a run-way of 40 in. instead of 37½. To cover this trough eight pieces of glass, 5 in. in length (and eight thermometers), were used instead of six as in the previous gradient (Fig. 3).

Ice remained in the flask for a period of 24 hr. However, when the ice was not in actual contact with the brass bar the temperature immediately went up at the cold end of the gradient. To avoid this a larger Thermos would probably be of advantage. Otherwise the gradient obtained was about the same as when an ice-box was used.

The chief advantages of this apparatus were as follows: (a) light in weight -- easily moved about by one person, (b) compact, took up less space, (c) used a minimum of ice. This gradient machine was not constructed until near the end of the preferendum work and no results from it are included in this paper.

4. TECHNIQUE OF EXPERIMENTS

(a) *Apparatus and working precautions*

So far as I am aware only one previous worker on thermo-preferendum has ever made any attempt to distinguish between floor temperature and air temperature in the gradient. Most have taken the air temperature by projecting thermometers through the top, or sides, of the apparatus while the insects remained in contact with a metal floor. In the present work it was decided to test the gradient for any possible differences in temperature within the apparatus itself. The following are the average results of twenty readings

taken at three different positions along the linear gradient, i.e. at 85 cm. the cold end; 55 cm. the centre of the gradient; and 10 cm. the hot end:

Position in gradient	Floor	Centre	Roof
85 cm.	6° C.	11° C.	11° C.
55	20.5	21	21
10	44	36	36

The average room temperature was 20° C. 'Floor' indicates that the thermometer was lying flat on the floor but the bulb was not in metallic contact with it. 'Centre', the thermometer bulb was projected through the glass cover, to about midway in the apparatus, in the same position as when experimental temperature readings were taken. 'Roof' indicates that the thermometer was propped tightly against the inside of the glass roof. All three thermometers (i.e. floor, centre and roof) were placed in tier one above the other at each of the three positions shown above.

As can be seen at the cold end the floor was 5° colder than the centre and roof. In the centre the floor, centre and roof temperatures were all about the same. This was more or less expected, since the temperature here was approximately the same as that of the room. At the hot end the floor was 8° hotter than the air above it or the roof. In no single reading was the difference between either the centre or air temperature and the roof ever more than 0.5°, the average being the same for both.

In order that the air surrounding the insects should be as nearly as possible at the same temperature as that recorded on the thermometers, and in order to avoid any controversy on the subject, a false floor was placed in the apparatus. This floor was of finely woven 'organdie' cloth and was held in place, half-way between the brass floor and the cover, by adhesive zinc-oxide plaster. This plaster had no apparent odour and was superior to all other sticking substances in preventing even the smallest insects from working their way beneath the cloth. It was later discovered that this false floor had the following additional advantages: (a) it was easy to see the insects against a white background, (b) the insects could easily get a foothold, (c) if any insects got on to their backs they could readily right themselves.

Thermometers were placed at 10, 25, 40, 55, 70 and 85 cm. In the early experiments temperature readings were taken every 30 min., but since there was little variation in the temperature the readings were reduced to one each hour in the later work. At each temperature reading a record was also taken of the room temperature. Since most of the insects experimented with had a preferred range of several degrees all temperature readings were taken to the nearest degree only, never to fractions.

It is impossible to have a temperature gradient without also having an inverse gradient in relative humidity, i.e. the relative humidity will be the lowest at the warm end of the gradient and highest at the cool end. The only

way to maintain a constant relative humidity in a temperature gradient would be by completely drying the air or by keeping it saturated. Since neither of these alternatives appeared feasible it was decided to measure and record the relative humidity each day. In this linear gradient it varied from 10 to about 45 %. However, as will be shown later, it was possible to keep an approximately uniform relative humidity when the experimental trough was filled with food for certain stored product insects.

Since the space above the false floor was only $\frac{1}{2}$ in. in height and 2 in. in width the relative humidity had to be taken with a very small instrument. For this purpose four Edney paper hygrometers were selected as used by Gunn & Kennedy (1936). These are watch-like instruments, 2 in. in diameter and about $\frac{1}{4}$ in. in thickness, and give a direct reading of relative humidity. They were placed in the apparatus at night, after the insects had been removed, and the relative humidity recorded the following morning. They thus had ample time to reach equilibrium before the readings were taken. The hygrometers were always placed at 0, 30, 60 and 90 cm. along the gradient. Each week the hygrometers were calibrated with a wet and dry bulb sling psychrometer. In addition, during the day, when not in use, they were kept in separate desiccators in which the relative humidity was controlled by known concentrations of sulphuric acid as described by Buxton & Mellanby (1934). Each hygrometer had a number, and the one used at the dry end of the gradient was kept at a low humidity (about 10 %); the one used at the moist end was kept at about 75 % relative humidity and the remaining two at intermediate humidities.

In very few of the papers on thermo-preferendum has the humidity ever been measured or even mentioned. Nieschultz (1933) made an attempt to overcome the humidity difficulty by having desiccating agents at the cool end and humidifying solutions at the warm end of the apparatus. Gunn (1934) forced a regulated stream of air of a known dew point into his apparatus. More recently Thomson (1938), working with mosquitoes, determined the humidity range to which they were insensitive and then kept his temperature gradient within this predetermined zone. However, with mosquitoes this was quite easy to do, since all stages were indifferent to a relative humidity between 30 and 85 %.

The hot water bath was kept heated by a gas flame day and night, and the ice-box was likewise kept continually packed with ice. A uniform gradient could thus be maintained continuously over a period of weeks or even months. The advantages of keeping the gradient going over long periods were: (a) one could commence work immediately upon arrival in the morning without waiting for a gradient to form, (b) the hygrometers could be left in the apparatus over night for recording the relative humidity at different positions along the gradient. The ice-box was packed three times a day, morning, noon, and night, with two parts finely crushed ice and one part common salt (sodium chloride).

This mixture gives a theoretical temperature of -18°C . according to Kaye & Laby (1921). An average of about 6 lb. of ice was used at each packing. In order to secure the maximum results from this freezing mixture it was necessary to keep the water level about 2 in. above the brass trough which projected into the ice-tin. The water above this level was periodically siphoned off into the sink. About once a month the salt which caked at the bottom of the tin also had to be cleaned out.

The first experiments were conducted in a laboratory with three south windows. Although the windows were shaded it was quite impossible to keep the light uniform on all parts of the gradient. During the same day the light might change from bright sunshine to cloudiness and at night to artificial light. The experimental trough was therefore kept completely dark by covering it with 'masonite' wall board cut to fit exactly over the glass plates. However, when the cover was lifted for the purpose of making an observation, the insects immediately became restless, and started moving about. After a little preliminary work, all apparatus and experiments were moved to a dark room in the basement. During each experiment only one small shaded light was alight, and this was below the level of the trough. An indirect uniform dim light, which was just sufficient to see the position of the insects, was cast over the gradient. This dark room had an additional advantage in that it was constructed as a constant-temperature room. Although no attempt was made to keep it at a uniform temperature it nevertheless remained between $18-21^{\circ}\text{C}$., night and day, throughout the year.

It should be mentioned that each time work was started with a different species of insect the gradient was thoroughly sponged out with cotton wool and boiling water, and the false floor removed and replaced with a new one.

(b) *Observations*

Each experiment was carried on for a period of 3 days. The number of observations made varied somewhat according to the nature of the experiment, but usually every half-hour the number of insects, and their positions in centimetres, were recorded. When insects were used with food, observations were made after they had been in the gradient for 1 hr., 2 hr., 4 hr., 8 hr., etc. This was to see how long it took the insects to come to their preferred temperatures and if their preference changed with time. After each experiment a control was carried out for 1 day at a uniform temperature. This was for the purpose of getting the random distribution of the animals when there was no gradient. A control often showed an insect to be strongly thigmotropic (for example, the bed-bug), or to have definite end preferences. So far, the Thomsens have been the only experimenters to use a control in connexion with preferendum work.

Thirty insects were usually used in each experiment, five being introduced into each of the six cover openings. Half-hourly observations usually resulted

in twenty-four readings a day, seventy-two in 3 days. Thus there was a total of 720 observations daily or 2160 for the 3 day experiment. The numbers of insects, both experimental and control, were all originally plotted against position in centimetres, and, superimposed upon this, the temperature and humidity were also plotted against position (Fig. 2).

Taking the insects' positions in centimetres rather than recording the number at each temperature had the following advantages: (a) it permitted the rapid recording of observations, and no interpolation between thermometers was necessary; (b) it allowed the plotting of experimental results

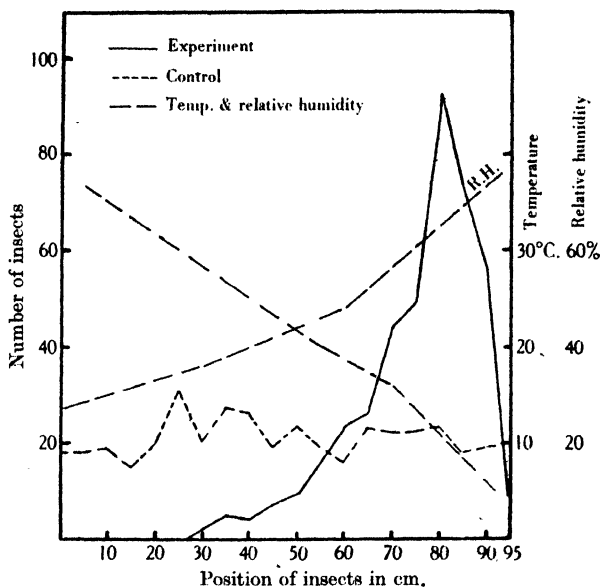


Fig. 2. Temperature-preference curve of adult *Apanteles congestus* (Braconidae, Hym.) arranged according to position in centimetres in the gradient. Each point is the average of not less than forty-two hourly observations on thirty insects previously kept at room temperature (cf. Fig. 3).

against a control—since the control was taken in centimetres at a uniform temperature, positions had to be taken in centimetres; (c) it is easy to convert position into temperature gradient, but more difficult to record the reverse process; (d) some insects may have an end preference which would not show up if positions were taken in °C.; (e) the results lend themselves more readily to statistical treatment; (f) some insects may not respond to a change in the temperature gradient; but if their positions were taken in °C. and the temperature gradient changed, they would appear to be changing their preference—when in reality it would be the temperature changing and not the insects.

If we have insects which distribute themselves evenly (i.e. no preference) in a gradient that is high at one end, low at the other and fairly uniform in the

centre, and if these insects were plotted against position, a straight line would result. If plotted directly against temperature, a curve would be obtained with a central mode, apparently indicating that these insects had a temperature preference for the centre of the apparatus, merely because a longer space at each temperature would be available in the middle. It would not show the even distribution of the insects nor that just as many per centimetre were at the hot and cold ends as were in the centre.

The difficulty of showing all results in this form is that each experiment requires a separate graph. When they are converted into numbers of insects against temperature this resulting graph is as shown in Fig. 3. This form has been used for a selection of results, as a larger number can be illustrated in a small space.

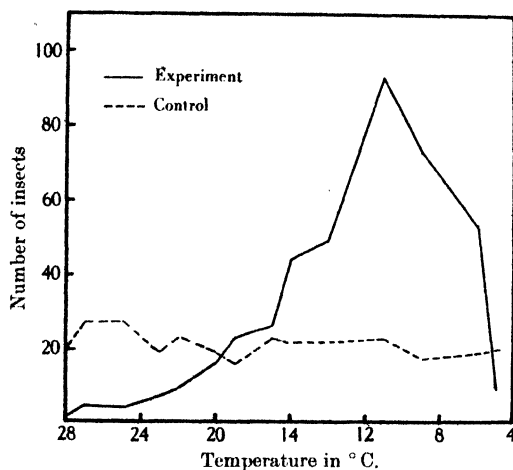


Fig. 3. Temperature-preference curve of adult *Apanteles congestus* (Braconidae, Hym. arranged according to temperature in gradient in °C. (cf. Fig. 2).

The original records were taken in the manner shown in Table 1. It is impossible to print all of these; but the original records are deposited at Rothamsted Experimental Station, where they can be consulted.

(c) *Insects used*

A total of twenty-three species of insects from six orders has been tested for their temperature preferendum. These have included insects of widely different environments and habits, such as the following: stored product insects, leaf-feeding insects, plant-sucking insects, human parasites, insect parasites, and soil insects. During the winter months work was done on stored product insects (mainly beetles), while in the warmer months insects from out of doors were used. The summer insects included such pests as ants,

Table 1. *Example of daily records kept for experiments using linear apparatus*

Exp. no. 36. Insect: <i>Apanteles congestus</i> . Stage of insect: adult. No. of insects: 30. Temperature previous habitat: 20° C.																																				
Cm. ...	Date Sept. 12	Time	° rel. hum.										Temperature °C.										Position of insects													
			33	40	50	73	35	30	25	20	15	10	20	85° Room	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90			
...	...	9 a.m.	35	30	25	20	15	10	20	5	.	5	.	2	3	4	1	.	2	4	.	5	6	2	7	.	5	
...	...	10 a.m.	35	30	25	20	15	10	20	2	2	3	.	.	3	3	1	8	.	6	2	.	.	.	
...	...	11 a.m.	35	30	25	20	15	10	20	1	.	.	1	2	.	3	.	3	5	2	5	.	5	3	.	.	.	
...	...	12 noon	35	30	25	20	15	10	20	6	.	1	.	3	6	2	4	2	6
...	...	1 p.m.	36	31	26	21	16	9	21	1	.	3	4	4	3	6	4	5	
...	...	2 p.m.	36	31	26	20	15	8	21	1	4	1	8	4	.	3	5	4	.	
...	...	3 p.m.	36	31	26	20	14	7	21	2	4	7	2	3	3	8	1	.	.	
...	...	4 p.m.	36	30	25	20	14	7	21	1	2	2	4	2	4	3	8	2	.	.	
...	...	5 p.m.	36	30	25	20	14	7	21	3	3	3	3	4	5	5	4	.	.	
...	...	6 p.m.	36	30	25	20	14	7	21	2	2	2	3	6	6	6	3	.	.	
...	...	7 p.m.	36	30	25	20	14	8	21	2	2	4	6	4	7	5	.	.	.	
...	...	8 p.m.	36	30	25	20	15	8	21	
...	...	9 p.m.	36	30	25	20	14	7	21	3	5	5	6	8	3	.	.	.
...	...	10 p.m.	36	30	24	19	13	6	21	3	7	5	11	3	1	.	.	.
...	...	11 p.m.	35	29	24	19	13	6	20	2	6	4	7	6	4	1	.	.
...	...		35.7	30	25	20	14.4	8	20.7	0	0	0	1	0	1	5	8	8	13	16	29	56	44	66	56	85	32	1	0							

wireworms, sawflies, earwigs, etc. The following is a detailed list of the species used.

Order	Family	Species
Dermaptera	Forficulidae	<i>Forficula auricularia</i> Earwigs
Hemiptera	Cimicidae	<i>Cimex lectularius</i> Bedbug
	Pyrrhocoridae	<i>Dysdercus howardi</i> Cotton stainer
Lepidoptera	Tineidae	<i>Tineola biselliella</i> Common clothes moth
Coleoptera	Elateridae	<i>Agriotes</i> sp. Wireworms
	Bruchidae	<i>Acanthoscelides obtectus</i> Bean weevil
	Ptinidae	<i>Ptinus tectus</i>
	Anobiidae	<i>Lasioderma serricorne</i> Tobacco beetle
		<i>Sitodrepa panicea</i> Biscuit weevil
	Curculionidae	<i>Calandra granaria</i> Granary weevil
		<i>Calandra oryzae</i> Rice weevil
	Dermestidae	<i>Dermestes vulpinus</i> Leather beetle
		<i>Anthrenus verbasci</i> Carpet beetle
	Tenebrionidae	<i>Tribolium confusum</i> Confused flour beetle
		<i>Gnathocerus cornutus</i> Horned flour beetle
		<i>Tenebrio molitor</i> Meal worms
	Cucujidae	<i>Oryzaephilus surinamensis</i> <i>O. mercator</i> Saw-toothed grain beetles
		<i>Laemophloeus turcicus</i>
		<i>Pteronidea melanaspis</i> Saw-flies
Hymenoptera	Tenthredinidae	<i>Apanteles congestus</i>
	Braconidae	<i>Acanthomyops</i> sp.
Diptera	Formicidae	
	Sepsidae	Sepsid flies

With many of the insects, both the immature and adult stages were tested separately and the results compared in an effort to find differences, or similarities, in their preferendum. With others the sexes were experimented with independently to see if sex could be in any way related to choice of temperature.

All species experimented with were kept at room temperature. As stated above this remained at 18-21° C. in the dark room. In addition to these, a number of insects were also kept at a high temperature (27° C.) to see if this would alter their preferendum. A constant temperature oven, thermostatically controlled to keep at 27° C., was used for this purpose. Some soil insects were

also kept at low temperatures (freezing) before being tested. A comparison was then made between those previously kept at room temperature and those kept at freezing point.

The technique of previous workers has been to place insects in a preferendum apparatus without food, in a strange environment and often in bright or changing light. (Thomsen & Thomsen (1937), who worked with maggots in dung, are an exception.) In an endeavour to determine if insects react to a temperature gradient in the same way with food as they do without it, several stored product pests were tried. The experimental trough was filled with their usual food (rice, bran, wheat, fish meal, etc.) and the insects distributed in the normal way. The glass cover to this linear trough was cut into six sections of 16 cm. each. This was a great advantage in removing insects with food, or with soil, since only two 8 cm. sections needed to be uncovered at a time. All twelve sections of food were removed at one time and placed in separate Petri dishes. This was to avoid any movement of insects, due to a change of temperature, when the apparatus was opened. This same technique was used for determining the position of soil insects along the gradient.

Since the thermometers projected directly into the food material, quite an accurate temperature record was obtained of the insect's actual habitat. An additional advantage in using food was that an almost uniform humidity was maintained over the period of the experiment (3 days). However, it is likely that if the food were allowed to remain in the gradient for a longer period a humidity gradient would have finally been set up.

In selecting specimens for an experiment, only the most active and healthiest ones were chosen. Those that were crippled, deformed or appeared abnormal in any way were thrown out. Each experiment was always carried through to its completion with the same individuals originally selected. For example, if one or two insects died, were lost or killed in the middle of an experiment they were not replaced by fresh ones. This accounts for the total number of observations not being the same in certain of the experiments. After the last observation was made at night the insects were removed from the apparatus and fed in a container separate from the stock supply. In transferring the insects to and from the gradient, they were always handled with a camel-hair brush in order to prevent any injury to them.

An equal number of insects, usually five, was introduced into each of the six thermometer openings. After each observation was recorded the insects were disturbed to see if they would return to a similar temperature. No attempt was made to direct them in any particular direction but just to start them moving. Two methods were used for this. The first was to have within the trough a light piece of bent iron wire which by means of a magnet could be used to disturb the insects from outside the glass cover, as used by Gunn & Cosway (1938). Secondly, insects that were not sensitive to the wire and magnet were disturbed by a thin copper wire, well protected with rubber.

5. RESULTS

(a) *Stored product insects* (Table 2)

The flour beetle (*Tribolium confusum*), when kept at room temperature, showed a definite temperature preference for 25 to 30° C. and gradually diminishing down to 10° C. (Fig. 4). In a separate experiment the beetles

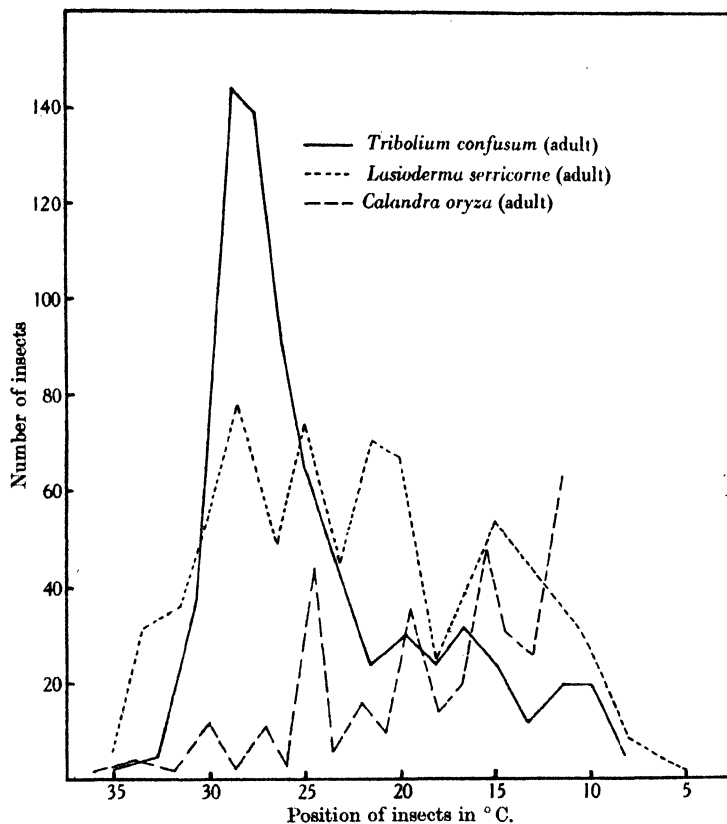


Fig. 4. Temperature-preference curves of adult insects of various species previously kept at room temperature (without food).

were kept for a month at a constant temperature of 27° C. (their optimum) previous to going into the gradient. These formed a very high peak at the cold end, 14° C., with the exception of a few that distributed themselves irregularly from 30 to 16° C. (Fig. 5). The peak number of insects thus occurred at a temperature 12° lower than when kept at room temperature. In general these results agree with the work of Bodenheimer (1928) on *T. confusum*.

In discussing extremes of temperatures Chapman (1931) makes the following statement: 'it would seem, in general, that the tropical insects, including

those of tropical origin, do not have the capacity for enduring dormancy. This is true of *Tribolium confusum*, which will die in a few weeks at 7° C.' In view

Table 2

Stage	Previous temp.	Food	Preferred temp. °C.	Preferred range humidity	Figure
<i>Tribolium confusum</i> (Flour beetle)					
A	Room	None	25-30	35	4
A	27° C.	None	(14)	60	5
A	Room	Bran	10-(25-30)	38	6
<i>Lasioderma serricorne</i> (Tobacco beetle)					
A	Room	None	15-32	40-55	4
A	Room	Rolled oats	8-32	45	6
A	27° C.	None	(15) and (27)	40-60	5
<i>Calandra oryzae</i> (Rice weevil)					
A	Room	None	10-20	40-60	4
A	27° C.	None	(14)-23	40-60	5
A	Room	Rice	8-(17)-25	60	6
<i>Calandra granaria</i> (Grain weevil)					
A	Room	None	(14)-25	20-60	—
A	27° C.	None	(14)	60	—
A	Room	Wheat	9-(20)-30	60	—
<i>Dermestes vulpinus</i> (Leather beetle)					
A	Room	None	(30)	60	7
L	Room	None	(30)	60	—
L	Room	Fish meal	20-30	55	—
A	Room	Fish meal	(7)-(16)-(25)	30-36:45	8
<i>Gnathocerus cornutus</i> (Horned flour beetle)					
A	Room	None	13-(20)-35	23-70	—
L	Room	None	13-(20)-35	23-70	7
L	Room	Bran	12-30	35	8
<i>Ptinus tectus</i>					
A	Room	None	(8)	80	—
A	Room	Fish meal	(8)	55	—
<i>Tenebrio molitor</i> (Mealworms)					
L	Room	None	13-(19)-20	25-42	7
L	Room	Bran	13-(19)-20	60	8
<i>Oryzaephilus mercator</i>					
A	Room	None	15-(24)-(28)	—	—
<i>Oryzaephilus surinamensis</i>					
A	Room	None	15-(24)-(28)	—	—
Replicate of above two					
A	Room	None	(10)-(28)	40-70	—
<i>Anthrenus verbasci</i> (Carpet beetle)					
A	Room	None	8-(12)-25	40-45	—
<i>Acanthoscelides obtectus</i> (Bean weevil)					
A	Room	None	13-30	15-40	—
<i>Sitropeda panica</i> (Biscuit weevil)					
A	Room	None	14-(21)	20-40	—
<i>Laemoploeus turcicus</i>					
A	Room	None	14-(17)-(21)-28	30-60	—
<i>Tineola biselliella</i> (Clothes moth)					
L	Room	None	14-(17)-20	30-40	—

A = adult; L = larva.

of the above the insects going from a previously high to the lowest possible temperature could hardly be associated with going to a more favourable habitat.

When given bran in which to move about and feed they still showed a marked preference for 25–30° C.; but there were also more insects at the lower temperatures and even a slight rise at the cold end (Fig. 6). *Tribolium*'s temperature preference in these two experiments corresponds quite closely to their optimum, which is 32° C. for reproduction, according to Chapman (1931).

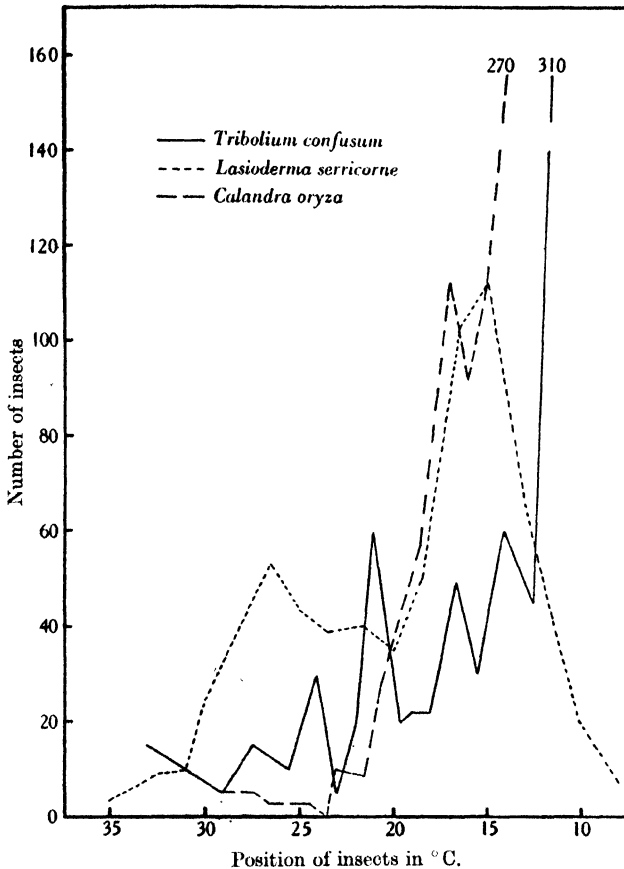


Fig. 5. Temperature-preference curves of insects shown in Fig. 4 but previously kept at a temperature of 27° C. (without food).

The temperature-preference curve for the tobacco beetle (*Lasioderma serricorne*) fluctuates over a zone from 32 to 15° C. (Fig. 4). When given food this zone became even wider, extending all the way from 32° C. to the cold end of the gradient (8° C.) (Fig. 6). However, after being kept at 27° C. for a month the beetle's preference was altogether changed. There was a slight peak at 27° C. and then a very decided one at 15° C. with a complete dropping off at the cold end (Fig. 5). In the first two experiments only about 25 % of the insects were found to be near 32° C.—the temperature given as their optimum

by Powell (1931)—and in the last experiment less than 15 % were near this figure.

Rice weevils (*Calandra oryzae*), previously kept at room temperature, present a violently fluctuating curve rising somewhat at the cool end (Fig. 4). When previously kept at a constant temperature of 27° C. they form a curve beginning at about 23° C. and rising very rapidly at the cool end (Fig. 5). On the other hand, weevils given rice, as a medium in which to choose their temperature, formed a peak at 17° C. which gradually sloped off to 25° C. on one side and 8° C. on the other (Fig. 6). Thus in every experiment these insects were found in greatest numbers at a temperature much below their optimum of 25° C., as given by MacLagan & Dunn (1936).

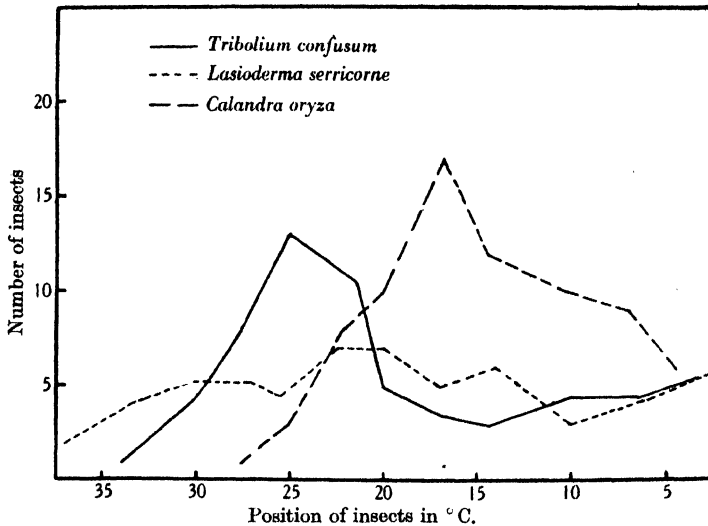


Fig. 6. Temperature-preference curves of insects shown in Figs. 4 and 5 when given food in the temperature gradient.

The temperature preference for the grain weevils (*Calandra granaria*) begins at 35° C., starts to rise at 25° C. and finally makes a very rapid ascent at 14° C., the cold end of the gradient. When the weevils were kept at a constant temperature of 27° C. for a month they gathered in even larger numbers at the cold end. Beginning at about 28° C. the numbers do not increase greatly until the temperature reaches 14° C., then the curve rises almost perpendicularly towards the cool end. When given food, the insects had a definite preference for 20° C., which tapered off to 30° C. on the hot and 9° C. on the cold side. With food, their preference was almost identical to that of *C. oryzae* above. However, in no case were more than about ten insects ever found near 28° C.—their optimum as found by Kunike (1936).

Both larvae and adults of the leather beetle (*Dermestes vulpinus*) behaved similarly in the temperature gradient. After reaching a very steep peak at 30° C. they dropped suddenly and then trailed off in small numbers to 9° C. (Fig. 7). With food the larvae had a rather wide preference zone, extending from 32 to 20° C. with another slight rise at 7° C. Adults with food had three

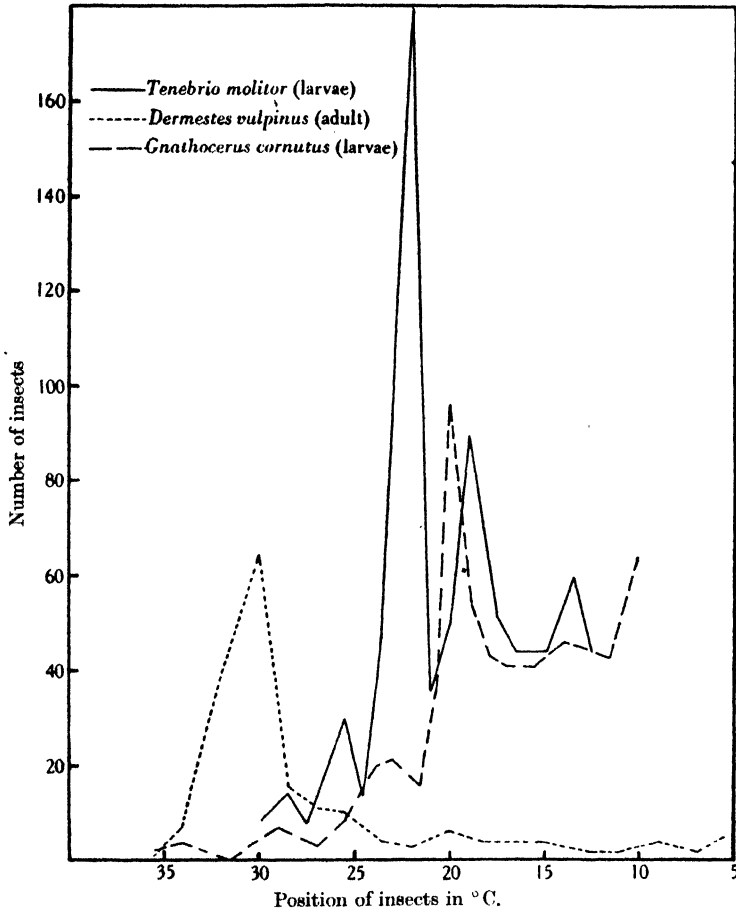


Fig. 7. Temperature-preference curves of certain adult and larval insects previously kept at room temperature (without food).

quite separate and definite peaks; the first one at 25° C., the second at 16° C., and the third at 7° C. (Fig. 8).

There was also a great similarity between the larval and adult temperature-preference curves of the horned flour beetle (*Gnathocerus cornutus*). They both began at about 35° C. and reached their maximum at 20° C., then declined to 13° C. However, the adults rose slightly at the end of the trough. When the larvae were placed in bran they occupied a longer range than before—a range

extending from 30° C. to about 12° C. (Fig. 8). No experiments were conducted with the adults in their food medium.

Both with and without food, *Ptinus tectus* showed a decided preference for the cold end of the gradient—about 8° C. Once, when tested without food, they showed a very weak preference for 25–20° C. *P. tectus* differed from most

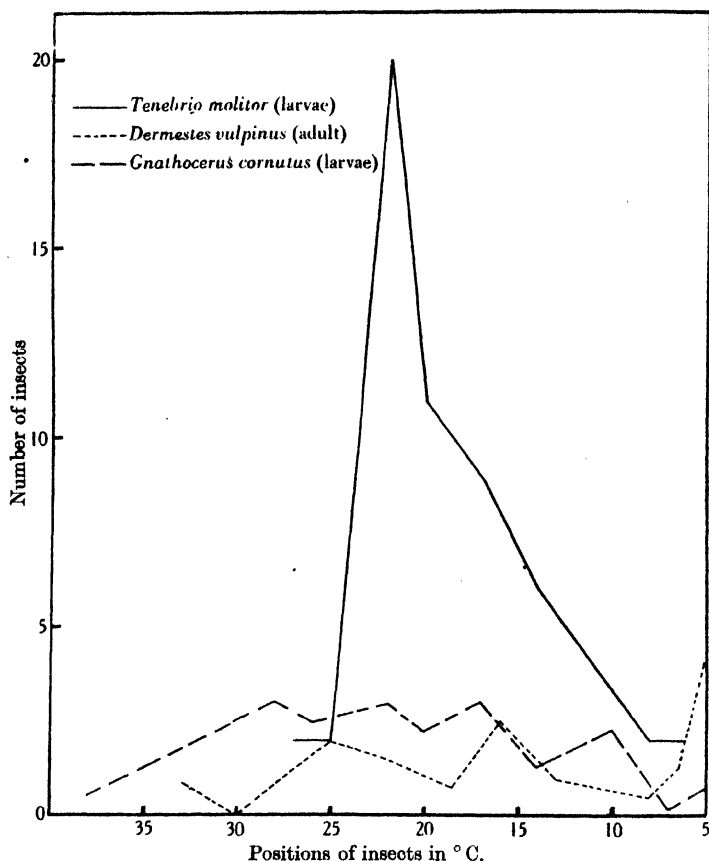


Fig. 8. Temperature-preference curves of insects shown in Fig. 7 but with food in the temperature gradient.

insects which went to a low temperature in that they were active and perfectly able to move about at all times. Most insects had to be removed from the cold end before they regained sensibility and could move about. It appears that this species remained in the cool zone because of preference and not because of being trapped. Also in the controls, at a uniform temperature, *Ptinus* distributed itself evenly throughout the length of the gradient.

Meal worms (*Tenebrio molitor*), both with and without food, rose to their highest peak at about 20° C. (Figs. 7 and 8). Without food, they had a minor

peak at 19° C. and another at 15° C. On the other hand, when food was supplied, there was a gradual decline from their maximum to 9° C. at the cold end. It might be suggested that the meal worms tested first left an odour, thus causing those in the second experiment to gather in the same place. This would hardly seem possible, since the first experiment was conducted on 19–21 April and the second on 29 November–1 December 1937. It is rather surprising that even more larvae were not found near the cold end, since they can survive cold much better than heat. Cotton & St George (1929) found that all stages of the meal worms were killed by a 1 hr. exposure to 52° C., while larvae exposed for more than 7 months to a freezing temperature remained alive.

A comparison between experiments with the saw-toothed grain beetles (*Oryzaephilus mercator* and *O. surinamensis*) showed the preference of both species to be almost identical, with two definite peaks. Here the two peaks were quite close together, one at 28° C., while the other was 24° C. The results for *O. mercator* were obtained from experiments conducted 5–7 March, while *O. surinamensis* was not tested until 19–21 March. In the presence of such striking similarities, it was decided to repeat the experiment at a later date. A replicate was started on 11 October, more than 6 months after the first experiment. Again the behaviour of the two species was similar, each showing two peaks. However, the two modes were more widely separated than before, the first being between 25 and 30° C. and the second at about 10° C. In both experiments the maximum peak occurred very near 27° C., the temperature given as the optimum for the most rapid development of *O. surinamensis* from egg to adult according to the investigations of Back & Cotton (1926).

The preference curve for the carpet beetle (*Anthrenus verbasci*) started at 30° C., attained its greatest height at about 12° C. and then suddenly dropped to almost nothing at the cold end. Its choice was thus decidedly within the cold zone, below 15° C., but not against the end of the trough.

The temperature preference for the bean weevil (*Acanthoscelides obtectus*) fluctuated quite violently between 30 and 13° C. All that can be gathered from results of this nature is that the weevils avoided temperatures above 30° C. In the control these insects had a definite preference for the ends. Biscuit weevils (*Sitodrepa panicea*) had one peak at about 21° C. and another at 17° C., with most of the insects in a zone between 21 and 14° C. Here again the insects showed an end preference in the control at a uniform temperature. Adult *Laemophloeus turcicus* presented a rather undecided temperature-preference curve, extending from about 30 to 14° C., with a peak at 21° C. and another at 15° C. Most of the insects were gathered in the zones 28 to 21° C. and 17 to 14° C. The clothes moth larva (*Tineola biselliella*) was the only Lepidopterous insect used. Here a definite preference was shown for the cool zone, 20 to 14° C., with a peak at about 17° C.

The above four experiments were conducted during the early part of this

work in one of the large entomological laboratories where uniform light and constant temperature did not prevail as in the dark room which was used in all other experiments.

(b) *Soil insects* (Table 3)

In all experiments with soil insects the thermometers projected directly into the soil, or other medium, in the same way as when food was used. At the hot end of the gradient moisture was continually condensing on the glass covers and dripping back on to the soil. As a result the moisture content of the soil remained fairly constant throughout. Most of the soil work was done with wireworms because (a) they are a serious agricultural pest in England, and (b) they could be obtained in sufficient quantity.

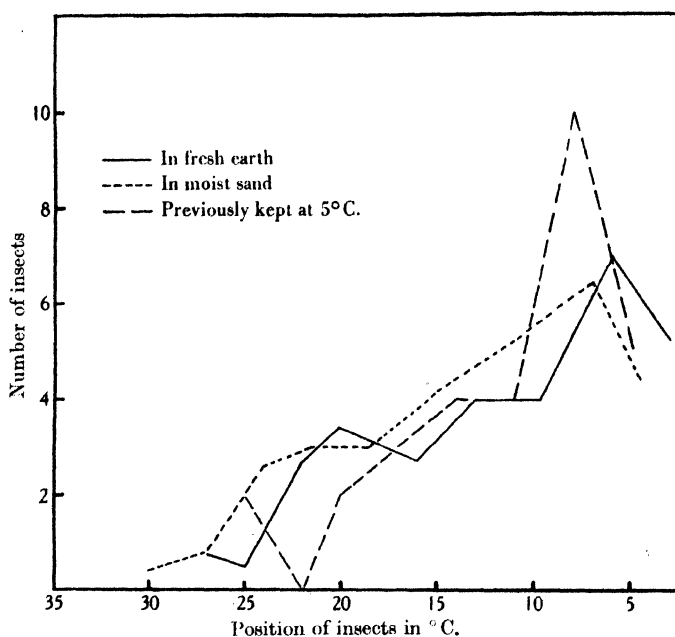


Fig. 9. Temperature-preference curve of wireworms, larvae of *Agriotes* sp. (Elateridae, Col.) under different conditions.

Fig. 9 shows a comparison between the results of wireworms that were placed in fresh earth (i.e. unmoistened) and those placed in well-moistened sand. As can be seen, about the only difference is that those in wet sand make a more gradual decline from a peak at the cold end (8-9° C.) of the gradient. In order to determine if previous temperature had any effect on their reactions a batch of wireworms was kept for 12 days at 5° C. After being placed in the gradient they were allowed ample time, 48 hr. to take up their preferred positions. From Fig. 9 it can be seen that the insects cover about the same range as when kept at room temperature. However, more individuals are

found at low temperatures, and there is a very abrupt dropping off from the cold end.

Ants (*Acanthomyops* sp.) were the second species of soil insects to be tried. They were also given fresh unmoistened earth and allowed to remain for 48 hr. before a reading was taken. In general their preference coincided with that of the wireworms, being highest at the cool end (8° C.) and gradually declining to about 30° C.

It is interesting that in none of the experiments with soil insects were they ever found to be most numerous at the very end of the gradient, their peak always occurring about 5–15 cm. from the cold end of the apparatus.

Table 3

Stage	Previous temp.	Medium	Temp. °C.	Relative humidity	Figure
<i>Agriotes</i> (Wireworms)					
L	Room	Fresh earth	(8)–20	—	9
L	Room	Moist sand	(8)–23	—	9
L	5° C.	Moist sand	(8)–18	—	9
<i>Acanthomyops</i> sp. (Ants)					
A	Room	Fresh earth	(10)–25	—	—
A	Room	None	(14)	30	—
<i>Apanteles congestus</i>					
A	Room	None	(10)–20	20–40	2, 3 and 10
<i>Pteronides melanaspis</i>					
L	Room	None	(11) and (31)	35 and 45	—
A (males)	Room	None	5–20	30–55	—
A (females)	Room	None	5–(8)–15	20–50	—
<i>Dysdercus howardi</i> (Cotton stainer)					
Nymph	Room	None	(12)–(25)	22–50	—
A	Room	None	12–25	22–50	—
Sepsidae (Diptera)					
A	Room	None	(9)	20	—
<i>Forficula auricularia</i> (Earwig)					
A	Room	None	(9) and 25–30	15 and 37–45	—
<i>Cimex lectularius</i> (Bedbug)					
A	Room	None	(12) and (22)	24 and 44	—

A = adult; L = larva.

Other insects (Table 3)

One day some small Braconid parasites were found in a cluster of spider's eggs. These were later identified by the British Museum as *Apanteles congestus* and proved to be one of the insects to give the most interesting results. The average for the 3 day experiment showed the parasites to have a decided preference for a temperature around 10° C. (Figs. 2 and 3). Here again the peak was 15 cm. from the end of the gradient, so one could hardly say that an end preference played a part in their reactions. It appeared that the insects' position shifted from day to day. The results were then plotted for each day separately, as shown in Fig. 10. On each successive day the parasites moved nearer and nearer to the cool end. The daily change in position is so definite that there can hardly be any doubt that they were actually moving to a cooler

region. An explanation as to why they successively moved from a warmer into a cooler region would be very difficult and could be a full-time problem in itself. Undoubtedly the Braconids underwent some fundamental physiological change.

At the same time that the experiment was started, eighteen surplus parasites, from the same egg mass, were placed in a vial and kept at room temperature. These were all dead after $1\frac{1}{2}$ days. After 3 days the experimental parasites, which were alive and quite active, were used in a control at a uniform

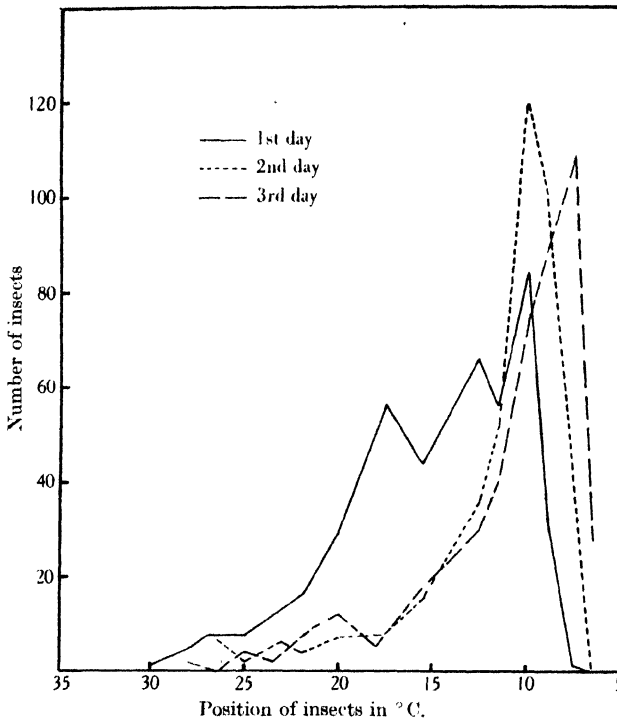


Fig. 10. Temperature-preference curve for *Apanteles congestus* for three successive days, showing the change in position towards the cool end of the gradient.

temperature. They gave a perfect random distribution throughout the gradient (Fig. 2). The methods followed in this experiment were slightly different from the normal. The parasites were not taken out of the gradient for feeding at night, but were allowed to remain in the apparatus continuously day and night. This was for two reasons: (a) the food of the Braconids was not known, and (b) the insects were too fragile to be handled any more than absolutely necessary.

A complete picture may be obtained of the behaviour of the saw-flies (*Pteronides melanaspis*), as the larvae can be compared with adults, and males

with females (Table 3). The larval preference went up very decidedly at 31° C., curved down, and then rose again at the extreme cold end of the gradient (11° C.). This is just opposite to the behaviour of the adult. Although they occupied the same temperature zone as did the larvae they rose in greatest numbers in the centre of the range (15–28° C.), rather than at the extreme temperatures near the ends of the zone. In comparing the differences between the two sexes it can be seen that they both extend over exactly the same range. However, the males are evenly distributed within this range, while the females are decidedly more numerous near the cool end (10–16° C.). The female saw-flies' choice for the second and third day was approximately the same, but for the first day greater numbers gathered at the lower temperatures.

Cotton-stainer nymphs (*Dysdercus howardii*) had a small peak at 29° C. and another at 25° C., but the majority were arranged between 25 and 12° C. The adults had a somewhat similar temperature range. These were tropical insects, imported from the West Indies, and one would expect to find more of them with a high temperature preference. Ants (*Acanthomyops* sp.), when given a choice of the gradient, without earth, repeatedly congregated directly against the cold end (14° C.). Those given soil, although they preferred the cool zone, were not found in large numbers tightly against the end, their peak appearing at least 15 cm. in from the end. Adult *Sepsid* flies present very much the same sort of preference as ants in an empty gradient, i.e. rising very sharply against the cold end (9° C.) of the trough. The end temperature here was about 5° lower than during the previous experiment.

The temperature preference for adult earwigs (*Forficula auricularis*) rises very suddenly at the cold end of the gradient. However, there is also a large number of insects gathered between 25 and 30° C. (Table 3). In this and the previous experiment the insects certainly did not gather at the cool end because of an end preference, as was readily shown by the controls. The bedbug (*Cimex lectularius*) preference, like that for the earwigs, goes up sharply against the cold end and also has a second peak near the centre of the gradient—about 22° C. (Table 3). However, the bedbug was not a suitable insect for experimental purposes, because its very strong thigmotropic tendencies were not easily overcome. The insects were inclined to gather in groups and when disturbed immediately formed another group regardless of temperature, except to avoid the very high temperatures.

6. DISCUSSION

It has been the purpose of this paper to show that, when given their choice of a temperature gradient, certain insects have a preferred temperature. The temperature range of all insects tested is shown and no effort has been made to select species to prove any particular case. It is realized that general conclusions cannot be drawn for insects as a whole from these experiments alone. However, it is believed that one conclusion is justified, namely, that insects

have a temperature preference, but it is a range rather than a point as suggested by some workers. The only preference some insects show is an avoidance of both extreme temperatures, e.g. the adult tobacco beetle when kept at room temperature, and horned flour beetle larvae in bran. On the other hand, they may merely avoid high temperatures and are to be found in all other parts of the gradient, e.g. the biscuit weevil, the bean weevil, rice weevil when kept at room temperature, adult *Dermestes* in food, and *Laemophloeus turcicus*. With insects that went to the cold end of the gradient it was difficult to distinguish whether they went there because of preference, or whether they wandered into the cold zone and were overcome, or trapped, before they could get away. Examples of such insects are the *Tribolium confusum* and *Calandra oryzae* when previously kept at 27° C., grain weevils, from both room temperature and 27° C., *Ptinus tectus* with and without food, ants and bedbugs.

Another rather peculiar preference is presented in the bi-modal curve with a peak near each end of the gradient. Saw-flies and earwigs are examples of this type. It has been suggested that these insects may have a wide range in which they are inactive, and are only active when they go outside of this zone. When they encounter too high a temperature they return to just within the upper limits of their preferred range. The same would happen when they encounter too low a temperature—they would return to just within the lower limits of their temperature zone. This is probably the explanation of a bi-modal curve with widely separate peaks.

In practice this would be what one might expect to find with most out-of-doors insects. It seems that it would be necessary for them to be indifferent to a fairly wide range of temperature in order to survive. Most summer insects must be able to withstand the hot days as well as quite cool nights. It is also not surprising that individuals of the same species sometimes give erratic and conflicting results in the temperature gradient when it is recalled that different individuals of the same insects, in nature, are often found at many different temperatures at the same time. Certain individuals may be in the hot sun while others are in the shade or other cool locations.

It might be said that insects go to a certain temperature in the gradient because they are attracted to the humidity there. The following insects have approximately the same temperature range both without and with food: *Dermestes* larvae, horned flour beetle larvae, meal-worms, tobacco beetles, rice weevils, *Ptinus tectus*, and *Tribolium* when previously kept at room temperature. Of course these are in addition to the soil insects, whose choice was also made independently of the relative humidity. The only insects that may have been trapped at the cold end were the ants.

One theory that may be advanced for insects gathering at the cold end of the gradient is they have no temperature preference, i.e. they were just wandering about, went into the cold zone, were overcome by the cold and

trapped there. In any case it is considered much more significant when insects show a preference outside of the cold zone.

No attempt was made to determine why an insect goes to or avoids a certain temperature. However, two theories which are worthy of mention are the classification of the insects' behaviour as follows: (a) behaviour reaction, in which insects receive the stimulus from a high or low temperature through certain receptors and their nervous system; (b) physiological reaction, in which the metabolic activities are speeded up, as when going into a higher temperature zone; or slowed down as when going into a lower temperature zone. In an insect that avoids a high temperature it would be impossible to tell whether it is showing a behaviour or a physiological reaction, without a detailed study of the insect's behaviour. On the other hand, an insect which avoids a low temperature is definitely showing a behaviour reaction. A study of the half-hourly and hourly observations from the daily record sheets shows that when insects leave the hot end of the gradient they invariably remain away from it. (After equal numbers have been introduced into the six thermometer openings.) However, they often move away from the cold end at first but eventually return to it and there remain.

It is possible that this temperature preferendum work may have definite practical applications as well as academic interest. Dr Baird, entomologist in charge of the Insect Parasite Laboratory, Belleville, Ontario, Canada, informs the writer that there all parasites, which are to be reared in large numbers, are tested for their temperature preference. They are then reared at their preferred temperature and much better results are obtained than formerly. It seems that the parasites do better at temperatures lower than those previously thought best for breeding purposes. The work has not yet been published. Temperature preference might also be used to advantage in eradicating insect pests, with a definite preferred temperature, from large warehouses and granaries where the temperature can be controlled. A small room, or other area, could be heated or cooled to the insect's preferendum while the rest of the building remained at an undesirable temperature. After the insects had gathered at their preferred temperature they could then be more easily destroyed.

During the course of the present work many questions and related problems have arisen to which a proper answer can only be given after further work and additional study have been carried out. One of the most important problems in connexion with temperature preferendum work, and one on which a large amount of work may be done, is: Why do insects seek out certain temperatures? What causes them to go to one temperature and not another a few degrees away? Another question almost equally important is: Why do certain insects, after being previously kept at a high temperature, have a lower preferred temperature than when previously kept at room temperature for the same length of time? In the future it is intended to concentrate mainly on the

preferendum of soil and aquatic insects. Here such variable factors as humidity, light and odour can be more readily standardized.

In the present experiment temperature has been considered the major factor in determining the position which the insects have taken up. However, such influences as humidity, odour, thigmotropism and possibly light must also be taken into account.

7. SUMMARY

1. A description is given of three different types of apparatus experimented with before selecting a linear brass gradient which gave a range of temperature from 10 to 35° C. in a straight-line gradient. This allowed the insects a choice of about 1° C. in every 4 cm. The relative humidity in such an apparatus varied inversely with the temperature except where food was used, when it remained practically uniform.

2. The temperature preferendum was tested of twenty-three species of insects from six orders. Insects were chosen to represent different environments or habitats, such as the following: stored product insects, leaf-feeding insects, plant-sucking insects, human parasites, insect parasites and soil insects.

3. Results are based on experiments each carried out for not less than 3 days. In every case a control was also run for 1 day to get the random distribution of the insects.

4. At the cold end of the gradient the metabolic activities of the insects were slowed down to such an extent that many species were trapped there, thus giving an apparent preference for the colder end. As a result when insects went to a warmer zone it was considered more significant than when they went to a cold zone.

5. The flour beetle (*Tribolium confusum*) when kept at 27° C. had a lower preferred temperature than when kept at room temperature.

6. Wireworms kept at 5° C. for a fortnight gave no different reaction from those kept at room temperature.

7. Insects that were given food in the gradient had a narrower preference zone than when not given food.

8. In general the preferences of the immature forms tended to coincide with those of the adults of the same species.

9. With saw-flies (*Pteronides melanaspis*) the males tended to have a wider temperature range than did the females.

10. The Braconid parasites (*Apanteles congestus*) went to a lower temperature on each successive day they were in the gradient and at the end of 3 days were alive and quite active. At the same time a surplus stock kept at room temperature were all dead at the end of 1½ days.

11. Generally speaking, all the insects experimented with have shown a definite temperature preference but the preference has been for a fairly wide range of temperature and not a point as has been suggested by some workers.

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PHYSIOLOGICAL RELATIONSHIPS BETWEEN INSECTS AND THEIR HOST PLANTS

II. A PRELIMINARY STUDY OF THE EFFECTS OF APHIDES ON THE CHEMICAL COMPOSITION OF CABBAGE AND FIELD BEANS

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(With 2 Text-figures)

THE difficulties of arriving at satisfactory estimates of losses due to the depredations of insect pests of crops have often been pointed out (e.g. Gimingham, 1939). In addition to the direct reduction of yields, insects may also be the cause of changes in chemical composition, a factor likely to be of particular importance in crops, such as sugar beet, grown as a source of special substances. Very little work has been done on this aspect of the problem. Leonard & Turner (1918) showed that the larvae of the beetle *Cerotoma trifurcata* Forst. reduce the amount of nitrogen fixed by the cow-pea by reducing the weight and nitrogen content of the root nodules. Hartzell (1913) studied the sugar and acid contents of Concord grapes obtained from vineyards infested by the grape leaf-hopper *Typhlocyba comes* Say. Grapes obtained from those sections of the vineyards sprayed with nicotine had higher sugar contents and lower acid contents than those obtained from unsprayed infested sections. Johnson (1934) carried out a detailed investigation of the effects of the potato leaf-hopper, *Empoasca fabae* Harris, on the chemical composition of alfalfa leaves. His results are compared below with those obtained in the present investigation.

BREVICORYNE BRASSICAE L. ON CABBAGE

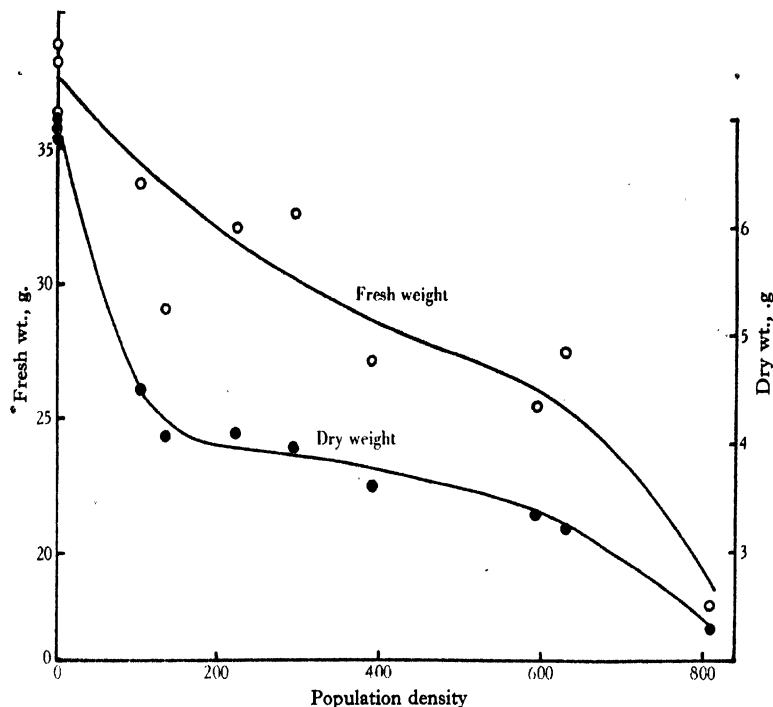
The effect of the cabbage aphid *Brevicoryne brassicae* L. on the chemical composition of cabbage (var. Sutton's 'Tender and True') has been chiefly studied. Material for analysis was obtained in the following way. Sixteen small cabbages were infested on 1 June 1937 with varying numbers (1-12) of aphides and four were kept uninfested as controls. Reproduction occurred chiefly on the small centre leaves. These were therefore cut out and left to wither in situ and the aphides then wandered on to the remaining leaves but did not produce an even infestation over the whole plant. The proportion of smaller leaves infested was not noticeably higher than that of the larger leaves. As some leaves on the plants were heavily and others only lightly infested, the plant was not prepared as a unit for analysis. Instead, the leaves were classified as highly, moderately, lightly and uninfested (control plants) leaves and three random samples of five leaves were collected of each type. The aphides were rapidly brushed off the leaves into alcohol for future enumeration and the leaves were weighed and dried to constant weight in an oven at 98° C. This technique is admittedly not as satisfactory as one which would involve analysis of the whole plant, but in a preliminary investigation its use is justified.

The degree of infestation is expressed by dividing the number of aphides (all instars) in

the sample by the fresh weight of the sample, the dividend so obtained being termed the population density, i.e. the number of aphides per gram of leaf.

Chemical analyses were carried out by methods already described (Evans, 1939).

Fig. 1 shows that there is a marked difference in the effect of increasing population density on the fresh and on the dry weights of the samples of cabbage. A reduction of



Text-fig. 1. The effect of population density of *B. brassicae* on the fresh and dry weight yields of samples of five leaves.

TABLE 1. *The effect of population density of Brevicoryne brassicae on the chemical composition of cabbage (expressed on a dry weight basis)*

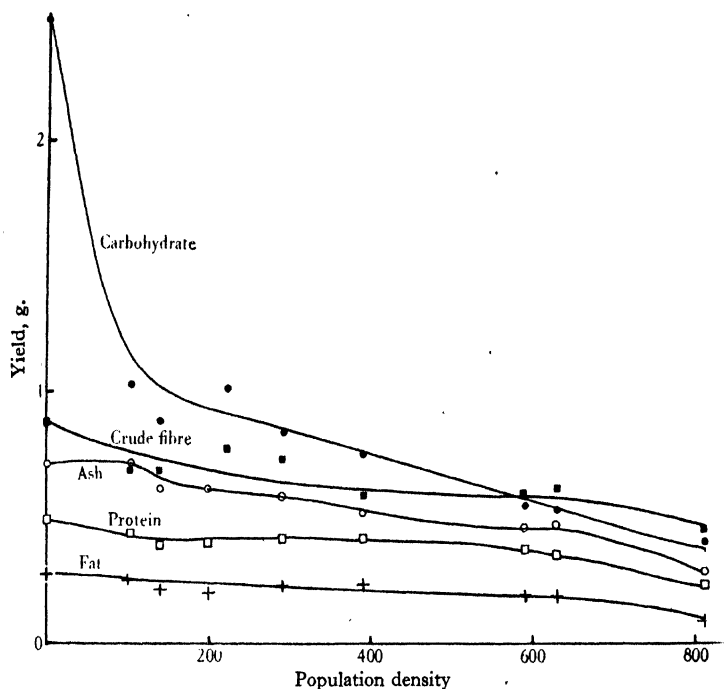
Population density	0	105	136	224	298	391	596	634	813
% dry weight	18.4	13.5	14.1	12.8	12.1	13.1	13.2	11.7	12.7
% ash	10.3	16.0	14.9	14.9	15.0	14.2	13.4	14.6	12.8
% fat	3.86	5.50	5.32	4.95	5.96	6.59	5.68	5.00	4.56
% protein N	1.12	1.52	1.49	1.58	1.67	1.81	1.78	1.68	1.56
% non-protein N	0.65	0.54	0.58	0.56	0.67	0.63	0.60	0.61	0.62
% reducing sugar	13.1	7.7	7.8	10.7	4.2	6.4	2.9	6.4	5.5
% sucrose	2.4	2.8	0.8	2.4	6.0	4.1	4.7	2.4	3.6
% starch	9.5	4.2	4.2	4.5	3.3	3.2	3.2	2.0	2.3
% acid-hydrolysable substances	11.0	8.3	8.3	7.2	7.7	7.1	5.4	5.9	5.8
% crude fibre	12.5	15.3	16.6	18.1	18.5	16.2	17.4	19.2	19.2

20% in the fresh weight is brought about by a population density of about 250 while a similar percentage reduction in dry weight is brought about by a population density of 50. This great reduction in the yield of dry material compared with that of fresh material is due to a sudden decrease in the percentage of dry matter in the infested plants (see Table 1).

Table 1 also shows the change in composition of the dry matter with increasing population density.

In general, the percentages of ash, fat and protein nitrogen increase to a maximum at a population density of 300-400 and then decline to a figure still higher than that of uninfested material. The percentage of crude fibre increases to a maximum at a population density of 600-800. The percentages of the several carbohydrate fractions decrease with increasing population density except perhaps that of sucrose, the figures for which are very erratic.

Fig. 2 shows the effect of these changes in chemical composition on the yields of carbohydrate (reducing sugars, sucrose, starch and acid hydrolysable substances), protein



Text-fig. 2. The effect of population density of *B. brassicae* on the yields of carbohydrate, protein, fat, ash and crude fibre.

($N \times 6.25$), fat, ash and crude fibre, obtained from each sample. The most noticeable effect is on the yield of carbohydrate which falls rapidly at first and then more slowly. The yields of crude fibre and ash fall fairly steadily with increasing population density while those of protein and fat are hardly affected by an increase in population density up to 400.

The effect of *Empoasca fabae* on the composition of alfalfa as found by Johnson (1934) is the opposite of that recorded above for *B. brassicae*, *E. fabae* causing an increase in the percentages of dry matter, reducing sugar, starch and acid hydrolysable substances and a reduction in the percentage of protein N (see Table 2). Johnson clearly showed that the feeding habits of *E. fabae* account for the increased carbohydrate and reduced protein nitrogen contents of attacked alfalfa. The leaf-hoppers, when feeding, puncture and block the conducting tissues in the petiole thus greatly impeding the removal of carbohydrates

from the leaves and the supply of nitrogenous salts to the leaves. With *B. brassicae* the cause of the great reduction in carbohydrate is not known. That it is perhaps due to a specific effect of a substance injected by the aphid on the mechanism of carbohydrate production is suggested by the fact that the production of fat and protein is affected but little by a low infestation which reduces carbohydrate production seriously.

TABLE 2. *Effect of Empoasca fabae on the composition of alfalfa. (Results calculated to the same bases as Table 1 from data given by Johnson (1934))*

	% dry weight	% reducing sugar	% sucrose	% starch	% acid-hydrolysable substances	% protein N
Not attacked	34.5	0.8	1.9	1.6	8.1	3.72
Attacked	37.0	3.4	2.7	7.2	16.6	2.55

APHIS FABAE SCOP. ON FIELD BEANS

Some data have also been obtained on the effect of *Aphis fabae* Scop. on the composition of field beans (Table 3). Material for analysis was collected on 2 Aug. from naturally infested crops of winter and spring beans. The plants were graded as clean, dirty and very dirty according to the appearance of the foliage since the aphides by this date had almost disappeared. In this case, aphid infestation has not materially affected the chemical composition of the beans. Similar figures were obtained for winter beans.

Thus it is seen from the instances recorded in this paper that insects may or may not affect materially the chemical composition of the plants on which they feed. A thorough knowledge of these effects might prove of value in assessing accurately the damage caused by pests.

TABLE 3. *The effect of Aphis fabae on the chemical composition of spring beans. (Expressed on a dry-weight basis)*

	Clean		Dirty		Very dirty	
% dry weight	23.6	24.9	22.7	26.9	19.2	23.6
% ash	4.3	4.4	4.6	3.9	5.3	4.6
% fat	3.2	2.9	4.2	3.7	3.8	3.9
% crude protein	23.3	22.8	24.5	24.1	28.9	25.4
% carbohydrates sol.	58.5	63.0	57.6	61.6	46.3	59.9

SUMMARY

Infestation of cabbage by the aphid, *Brevicoryne brassicae*, caused a marked decrease in the amount of carbohydrate synthesized but smaller decreases in fat, crude protein and other constituents. Infestation of field beans by the aphid, *Aphis fabae*, did not have any great effect on the chemical composition of the crop.

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STUDIES ON THE BRITISH WHITE-FLIES (HOMOPTERA, ALEYRODIDAE)

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WITH SEVEN TEXT-FIGURES.

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Introductory.

LITTLE has been added recently to the knowledge of the British ALEYRODIDAE with respect to their morphology, habits and food-plants. Their systematics have been equally unsatisfactory, and since the descriptions were inadequate in affording positive diagnostic characters, the position of some species was as undefined as it was about fifty years ago.

Since the correct determination of a species is important to the entomologist, and a knowledge of its biology is essential before undertaking control measures, these investigations were made to throw some light on the available species.

The comparative morphology was studied and some of the previous specific characters have proved to be of little significance. The identity in some of the species was established by their structural uniformity as well as by transference to alternative host-plants. These when successful confirmed the identity and also eliminated the possibility of the existence of biological food races. Two new species have also been described from ferns in the Royal Botanic Gardens at Kew (Trehan 1938).

Observations have also been made on parasites and parasitisation in some of

the species, and cross inoculations were tried to breed a parasite from one host on another.

The present investigations were continued from October 1936 to May 1938, under the supervision and guidance of Dr. C. B. Williams, the Chief Entomologist at the Rothamsted Experimental Station.

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I consider it my privilege to express my deepest gratitude to Sir John Russell, the Director of the Institution, for the facilities provided even to the extent of the use of his private garden and plants for field experiments. I am equally indebted to Dr. C. B. Williams for his keen interest in the problem and for going through the manuscript; to Dr. H. F. Barnes and Dr. A. C. Evans for their general assistance.

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The generosity and the encouragement of the Indian Central Cotton Committee by the grant of a foreign scholarship for higher study, is also highly appreciated.

Review and comments on some of the previously adopted characters and the significance of those now adopted.

Previous authors based specific differentiation on certain variable characters such as the food-plant, colour and shape of the body, and number of hairs and proportion of the antennal segments, but it has been now ascertained that the variations common in such characters were totally ignored: therefore, such criteria have often led to synonymy and to no organised system of classification. Prior to the standard work by Quaintance and Baker (1913-14) morphological characters in ALEYRODIDAE were not studied thoroughly. Burmeister (1835) and Westwood (1840) considered the antennae to be of six segments. Ruricola (1846) even doubtfully stated them to have five segments. Koch (1857) noted the parameres (forceps), which are a stable character in the family, for the first time in the male of *A. lonicerae*. Signoret (1868) gave a curious description of lingula which he noticed only in the adult of *A. rubi*—"tubercle more or less elongated and vesicular visible in both sexes, more in female" [translated]. Douglas (1891 and 1895) gave an interesting interpretation of the vasiform orifice in the pupae of *A. rubicola* and *A. carpini*.

The vasiform orifice is a most specialised organ in this family and exists in all stages after the egg. Maskell (1895) first recognised its importance and stated "... I have noted specific variations in this organ and believe it quite a valid differentiating character." Peal (1903) also considered it a strong character for the species. Quaintance (1908) stated "these structures vary much with different species and consequently furnish valuable diagnostic characters." Quaintance and Baker (1913-14, 1917) and Baker and Moles (1921) emphasised the importance of this organ in classifying the genera of the world.

The genitalia in insects are also recognised to possess certain permanent features which differ with the species. Kirkaldy (1907) regarded the genitalia in the male as the final test of a species and even as affording generic criteria. Muir (1916) placed considerable significance on the aedeagus and the male genitalia of DELPHACIDAE. Giffard (1921) remarked that "because of their

constancy and reliability as a specific character these organs are unquestionably of first importance to the student in making correct determinations." Singh Pruthi (1925) stressed the importance of these organs in the classification of JASSIDAE. Snodgrass (1931) considered these organs to give the best characters by which species may be distinguished, and, similarly, Delong and Davidson (1937) considered the chitinous parts of the genital chambers as the most important structures for the separation of species.

Attempts were therefore made during the present investigation to make a comparative study of both the vasiform orifice and genitalia which have given quite significant differences. It is therefore believed that these organs give far more reliable specific differences than any other characters. In certain cases, however, some other characters may also contribute some supplementary evidence.

Technique.

For descriptive purposes the stages of the various species were reared under observation but occasionally were also obtained in the field, and mounted in Berlese's fluid. For the study of the genitalia and finer details the specimens were treated with 10% KOH solution and then neutralised in 2% solution of acetic acid in 90% alcohol and ultimately mounted in balsam.

Aleyrodes loniceræ Walker 1852 and *A. rubi* Signoret 1868.

Walker (1852) described *A. loniceræ* from *Lonicera periclymenum* (Honey-suckle), giving a brief account of the coloration of the adult and pupa. Koch (1857) described the same species, but the colour of the adult does not agree with the original description. Frauenfeld (1867) supported Walker and stated Koch's *loniceræ* is not Walker's *loniceræ*. Signoret (1868) disagreed with Koch in considering the "forceps" in any way specific to this species. At the same time, he laid considerable stress on the number and distribution of the dorsal spines on the larva. Douglas (1896) contributed for the first time a description of its pupa. His description included a brief mention of the size and shape of the "triangular formation," which is most probably the vasiform orifice. Further, he doubted Walker's description of the colour of the pupa and described it as deep golden yellow.

Since then the species has remained neglected except for a few casual references. Quaintance and Baker (1914) assigned to it a generic position under *Aleyrodes*, and Harrison (1920, 1931) and Marriner (1931) gave an account of its distribution in the north of England. Deshpande (1933) published a brief account of the various stages of this species and showed that the eyes in *A. loniceræ* are similar to those in *A. rubi*. Further, he described two kinds of pupae in *A. loniceræ*—one with the dorsum apparently smooth and the other with six pairs of dorsal spines. The colour of the pupa is described as "olive-yellow." Deshpande also stated that, "in this species there are also specimens which are quite yellow without any dark colour on the body except one black mark on the wings. But there is no difference in the general structure."

Signoret (1868) described *A. rubi* from *Rubus fruticosus* (Blackberry) and gave a detailed description of the colour of the adult and a short account of the larva. It was remarked that the distinguishing characters between these two species (*A. loniceræ* and *A. rubi*) exist in their larvae. He stated, "The larva resembles that of *A. loniceræ*, showing the same bristles at the same place, only on the median line one observes at each abdominal segment, a distinct area (impression) which is more visible at the base than at the apex" [translated].

On the other hand, Douglas wrote of *A. lonicerae* "... the segmentation of the abdomen is evident, also there is a median row of concolorous raised spots." Evidently there appears to be a different interpretation of one and the same fact.

The table below summarises the descriptions of these species by the respective authors, from which it will be evident that there is little appreciable difference on which to consider them as distinct species. Signoret, while describing *A. lonicerae*, missed the item 9, which otherwise was regarded as quite a distinctive character in separating the species. Douglas, however, cleared up the doubt by pointing out this feature in *A. lonicerae* (Table I).

TABLE I.

Brief remarks from the descriptions of *A. lonicerae* and *A. rubi*, by Douglas and Signoret.

<i>A. lonicerae</i> (Douglas)	<i>A. rubi</i> (Signoret)	<i>A. lonicerae</i> (Signoret)
1. Abdomen yellow.	1. Yellow, spotted with blackish-brown.	1. Yellow, spotted with blackish-brown.
2. Head black, yellowish at sides.	2. Head almost entirely black, cheeks yellow.	2. No mention.
3. Thorax grey-black, margins pale.	3. Thorax and chest more or less black, sutures more or less pale.	3. Black spots on pro- and meta-thorax, base of abdomen blackish.
4. Eyes brown, divided.	4. Eyes reddish-brown, very divided.	4. No mention.
5. Antennae pale brownish.	5. Antennae brownish.	5. No mention.
6. Thighs black, extremities yellowish, tibiae yellowish, extremities black, tarsi black.	6. Feet black except the articulations more or less pale.	6. No mention.
7. Wings white with one grey spot in elytra at the end of the median nervure.	7. Elytra with a single black spot.	7. Single spot in elytra at the apex of the vein.
8. Larva with six pairs of hairs.	8. Larva: same arrangement of bristles as found in <i>A. lonicerae</i> .	8. Larva with six pairs of bristles.
9. Larva with concolorous median row of round spots.	9. Each abdominal segment in larva has an "impression" on the median line.	9. No mention.

The supposed differentiating characters between the so-called distinct species, therefore, were regarded as their food-plants and as a minute structural difference in the larvae. Moreover, colour and the description of the pupa in *A. lonicerae* have been the subject of considerable controversy and conflicting opinions have been expressed. It is now known that this may be the result of collecting specimens at different times of the year.

Present Observations.

To verify the supposed structural differences in both species, their morphology was studied critically and the biology investigated both in the field as well as in the laboratory. The distinct areas stated to be present in the median abdominal line of the pupa in *A. rubi* are also met with in the pupa of *A. lonicerae*.

Pupae of two types have been observed in nature: (1) white, (2) yellow or even tending to golden yellow. Therefore, the description of the pupa by Walker is not wholly incorrect. His description was probably based on the examination of a few specimens, particularly from the summer brood, and thus a complete range of variations was not studied. At the same time, the descriptions by Douglas and Deshpande were also based on restricted observations.

As regards the presence and distribution of dorsal spines on pupae, Douglas remarked "the dorsal hairs of the larva have mostly disappeared but a few of them are persistent in some specimens." The statement is true, but is unsatisfactory because a complete range of variations in this respect was not studied. Similarly the views expressed by Deshpande as to the two kinds of pupae require further explanation. Apart from the specimens falling in group no. (1), no strict arrangement in the number of spines has been observed. On the contrary, considerable variation has been noticed in their number from an apparently hairless form to a full complement of seven pairs of spines. This, however, is not contrary to expectation as it has previously been noticed in *B. gossypiperda* by Husain and Trehan (1933) and by me again in *A. carpini*.

The present observations may be summarised thus :—

1. *Colour of the pupa* (*A. loniceræ* type).

(A) White—very common during summer, after which the number decreases considerably.

(B) Yellow—tending to golden yellow—during autumn, winter and early spring; almost absent in summer.

2. *Number and distribution of dorsal spines* (Table II).

- i. Full complement of seven pairs—1 cephalic, 3 thoracic, 2 abdominal and 1 vasisformal.
- ii. Six pairs—abdominal pair single.
- iii. Five pairs—one thoracic pair missing; occasionally asymmetry may be noticed.
- iv. Four pairs—abdominals missing.
- v. Three pairs—only a single thoracic pair and the abdominals missing.
- vi. Practically hairless—a few minute hook-like spines visible.

The arrangement cited above equally describes the pupae on blackberry—hitherto known as *A. rubi*, except for the condition mentioned under vi.

TABLE II.

Variations noted in the number, distribution and development of dorsal spines in *A. loniceræ* (III instar and pupa) on respective food-plants.

Instar	<i>A. loniceræ</i> on Honeysuckle (<i>Lonicera periclymenum</i>)					Instar	<i>A. loniceræ</i> on Blackberry (<i>Rubus</i> sp.)				
	Pairs of dorsal spines						Pairs of dorsal spines				
	Total no. of pairs	Ant- tennal	Thor- acic	Abdo- minal	Vasi- formal		Total no. of pairs	Ant- tennal	Thoracic	Abdominal	Vasi- formal
III	3	1++	0	1++	1+	III	3	1++	0	1++	1+
	3	1+	0	1--	1--		3	1++	0	1+	1+
	3	1--	0	1--	1--		4	2++	0	1++	1+
	3	1+	0	1+	1--		5	2++	0	2++	1+
	4	2+	0	1+	1+		9	2++	3++	2++	2+/++
	5	2+	0	2-/+ ¹	1+						
Pupa	3	1++	1-	0	1--	Pupa	3	1++	1++	0	1++
	3	1--	1--	0	1--		4	1+	2+	0	1+
	4	1++	2-/+ ¹	0	1-		5	1++	3-/+ ¹	0	1+
	4½	1++	2++ ²	0	1++		5½	1++	3-/+ ¹	½ ²	1++
	5	1++	3++	0	1++		6	1++	3++	1++	1++
	6½	1++	3½++ ²	1+	1+		7	1++	3++	2+/++ ¹	1++

— Minute. -- Very minute. + Developed. ++ Well developed.

¹ Asymmetry in development. ² Asymmetry in numbers.

The adult of this species has always been described as grey in colour. During the present investigations, however, two types of adults were met with : (1) yellow, without any markings on the body except a black spot on the wings, (2) grey, showing considerable dark brown markings on the body besides those on the wings. The seasonal colour variations have been described by Trehan (1939).

The morphology of all the stages of *A. lonicerae* was studied and compared with that of the respective stages of *A. rubi*. The general structure in both these forms agrees in all details and the two are absolutely inseparable morphologically.

The previous differentiating characters have been proved to be of least significance because of the variations in dorsal spines as well as in the colour of the pupae and adult. The identity of the two forms is concluded on the evidence of the identical nature of the vasiform orifice in their respective stages as well as by the genitalia. The characteristic notch towards the distal end of the parameres with its claw-shaped ending and the heel-shaped structure sub-apically are quite identical in both the forms. In the female also the genitalia are alike.

This was further supplemented by the comparative study of other characters such as the eyes, antennae and wings of adults. All these characters agree remarkably in their structure in both forms. Nevertheless, some variations have been observed in the measurements of the antennal segments in different sexes as well as in individuals, but these were equally applicable to both the forms. For instance, segment VII is the longest of the distal four segments, but variations have been noticed in its measurements and occasionally segment V approaches it in length. Because of the pronounced variations, specific differentiation is not justified on superficial characters.

Biology and breeding tests.

Biology. The first record of an adult on honeysuckle was on 1.vi.1937, from Batford, Herts. The body colours were fully developed. The adults were quite scarce after that, but a few eggs were also noticed on honeysuckle, at Bricketwood, Herts, on 15.vi. In the first week of July, at Batford, Herts, besides a number of nymphs in the 2nd and 3rd instar, 8 pupae and 7 adults were also collected from honeysuckle. These adults were of the yellow type and the presence of a few pupa cases indicated that the first generation was over. The pupae were of white colour and the adults which emerged from them, in the laboratory, were also yellow. Since then both the pupae and the adults were frequently met with on honeysuckle—the adults being yellow and the pupae white.

Towards the middle of July eggs were noticed both on honeysuckle and on blackberry, though relatively fewer on the latter. Comparative counts on the 22.vii. gave 339 eggs on 60 leaves of honeysuckle against 103 on 40 leaves of blackberry. An average leaf of blackberry is, however, relatively larger than one of honeysuckle. The number of eggs laid on individual leaves varied from 1 to 9 on blackberry and 1 to 21 on honeysuckle. The adults of the yellow type were common on both the host-plants.

It is presumed that feeding of the first brood commences on honeysuckle, from where the pest migrates to blackberry. The relative infestation in nature, therefore, was found to be higher on honeysuckle. The probable cause of this variation in infestation is in the relative difference in the leaf area of the respective hosts, or the result of some constitutional changes in them as previously studied by Husain, Puri and Trehan (1936).

Series of observations on the sex ratio during July to November, 1937, at two widely separated localities gave the following figures :—

	Batford	Bricketwood
Total catch	303	403
Females	210	288
Males	93	115
Percentage of females	69.3	71.4

Average percentage of females 70.5.

The adults are long lived, and therefore the generations overlap. During November, only females were observed in the field. During autumn the adults were frequently observed taking shelter under the leaves of small oak bushes. In no case, however, was any egg or nymph observed on them. It is therefore presumed that such a coincidence probably led Signoret to describe the adults of *A. quercus* (from oak) as grey and the description practically corresponded with that of adults of *A. loniceræ*. A few overwintering females from the laboratory stock when shifted to a hot-house during March and April 1938, laid eggs readily. It is, therefore, quite probable that such individuals may start oviposition in nature as soon as the favourable period approaches. Overwintering in the pupal stage is also common.

Life-history. In nature egg-laying is very heavy during the summer but decreases considerably towards the end of September and may even stop later. The eggs, as a rule, are laid singly. The area frequented by the adult becomes conspicuous by the presence of a white waxy powder in the centre of which lies an egg which is also dusted profusely. Occasionally, however, two eggs are noticed close together. The females are easily disturbed when in the act of oviposition. It has been repeatedly observed that the eggs may not turn brown before hatching.

TABLE III.

Life-cycle and cross inoculations of *A. loniceræ*, on alternative food-plants, 1937.

Adults from	Life-cycle completed on	Date of introducing the adults	Earliest date of hatching of eggs	Earliest date of 1st moult	Earliest date of pupation	Earliest date of emergence	Shortest duration of the life-cycle in days
<i>Lonicera periclymenum</i> ⁷	<i>Lonicera periclymenum</i>	1937 1.vi. ¹	1937 12.vi.	1937 —	1937 —	1937 —	—
<i>Lonicera periclymenum</i>	<i>Lonicera periclymenum</i>	6.vii.	19.vii.	1.viii.	23.viii.	29.viii.	54 ⁴
<i>Symphoricarpos racemosus</i> ⁸	<i>Symphoricarpos racemosus</i>	6.vii. ³	20.vii.	3.viii.	25.viii.	1.ix.	57 ⁴
<i>Rubus fruticosus</i> ⁹	<i>Lonicera periclymenum</i>	9.vii.	21.vii.	3.viii.	26.viii.	29.viii.	51 ⁴
<i>Lonicera periclymenum</i>	<i>Symphoricarpos racemosus</i>	13.vii. ³	27.vii.	9.viii.	—	10.ix.	59 ⁴
<i>Lonicera periclymenum</i>	<i>Symphoricarpos racemosus</i>	16.vii. ³	3.viii.	—	—	8.x.	84 ⁵
<i>Lonicera periclymenum</i>	<i>Rubus fruticosus</i>	23.vii.	4.viii.	10.viii.	—	8.ix.	47 ⁶
<i>Rubus fruticosus</i>	<i>Lonicera periclymenum</i>	30.viii.	14.ix.	22.ix.	12.x.	19.x.	50 ⁶
<i>Lonicera periclymenum</i>	<i>Rubus fruticosus</i>	31.viii.	15.ix.	—	12.x.	19.x.	49 ⁶
<i>Lonicera periclymenum</i>	<i>Lonicera periclymenum</i>	9.ix. ³	—	—	—	21.x.	42 ⁶

¹ The plant as it was transplanted, dried up, and the nymphs did not develop into adults.

² Breeding was on plants growing in nature, in a very shady corner of a garden.

³ In hot-house.

⁴ Parents yellow, offsprings : males yellow, females grey.

⁵ Parents yellow, offsprings all grey.

⁶ Female parents grey, offsprings all grey.

⁷ Wild honeysuckle.

⁸ Snowberry.

⁹ Wild blackberry.

The species is multi-brooded and the generations overlap. During 1937, the shortest duration of the egg stage, observed under field conditions, varied from 11 to 18 days. The shortest duration of the nymphal stage was 27–36 days and that of the pupa 6–7 days (Table III).

Cross inoculation. The adults bred from the pupae on respective food-plants were sleeved on alternative food-plants. Thus the adults from honeysuckle were bred on snowberry and blackberry as well as those from blackberry on honeysuckle. To verify the duration of the life-cycle, the adults were also bred on their respective food-plants. These inoculations were quite successful and the life-cycle durations were almost unaffected, as is shown in Table III.

Alternative hosts. The adults have been collected from oak and hazel in large numbers and even from strawberries during autumn. None of these, however, is regarded as a true alternative host because immature stages were not obtained on them in the field.

During March and April 1938, the following observations were made in a hot-house :—

(1) Adults of *A. loniceræ* were sleeved on strawberry, where eggs were laid and the nymphs flourished very well. The life-cycle was completed in about 47 days.

(2) Adults of *A. loniceræ* were allowed to breed on oak leaves. Most of the adults were found dead in about three days. In one case eggs were not laid; in another only two eggs were laid, while in the third case five eggs were noticed. These eggs did not hatch.

This species has been observed breeding freely on snowberry¹ (*Symphoricarpos racemosus*) and on french-willow herb (*Epilobium angustifolium* L.). These plants, therefore, are an addition to the list of the known food-plants.

Description of the stages of Aleyrodes loniceræ Walker (figs. 1, 2).

Egg. Oblong, pedunculate, chorion smooth; yellowish-white, dusted profusely with white waxy powder, average measurements 0.27×0.10 mm.; stalk about 0.03 mm.

Nymph 1st instar. Elliptical, pale yellow, margins surrounded by a waxy fringe; average measurements 0.35×0.20 mm. Marginal spines sixteen pairs; dorsal spines three pairs—antennal, abdominal and vasiformal, only the antennal pair slightly better developed. Occasionally, however, more spines may be met with; ventrals 3 pairs—cephalic, rostral, and vasiformal. Antennae three segmented, 3rd the longest of all and provided with two spines, average length about 0.09 mm. Eyes divided; legs functional. Vasiform orifice subcordate (fig. 1), average measurements 0.03×0.04 mm., inner lateral and caudal margins not thickened, posterior margin slightly notched; operculum almost rectangular, measuring, on an average, 0.01×0.03 mm., caudal margin with a few minute hairs; lingula thick and almost cylindrical, setose with 4 prominent hairs shooting out from the distal half, length 0.02 mm. and more than $\frac{1}{2}$ exposed beyond the operculum.

Nymph 2nd instar. Pale yellow; oval, margin lightly dentate, surrounded by a narrow waxy fringe, average measurement 0.52×0.32 mm. Marginal spines three pairs, the anals prominent; dorsal spines 2–6 pairs; ventrals 1–4 pairs, generally minute. Eyes entire; legs degenerate; antennae atrophied, directed backwards, average length 0.02 mm. Abdominal segments marked by a series of concolorous tubercles on either side of the median line. Vasiform orifice subcordate, average measurements 0.04×0.05 mm.; inner lateral margins slightly roughened, caudal margin slightly notched; operculum sub-rectangular, lateral margins curved, average measurements 0.02×0.04 mm.; lingula short and thick spatulate, setose all over and armed distally with a pair of long hairs (fig. 1), average length 0.02 mm.; about $\frac{1}{2}$ exposed beyond operculum.

¹ Dr. C. B. Williams thinks that he has seen an old record of a white-fly on snowberry, but the reference was not traced.

Nymph 3rd instar. Shape and colour as in the previous instar; average measurements 0.73×0.50 mm.; abdominal segments marked by a series of concolorous tubercles on either side of the median line. Marginal spines three pairs—the anals being prominent; dorsal spines 3–4 pairs, variable; ventrals 1–2 pairs, minute. Eyes entire; legs degenerate; antennae atrophied, directed inwards, ending distally in a hook. Vasiform orifice subcordate, average measurements 0.05×0.06 mm.; inner lateral margins slightly roughened, and the caudal margin notched; operculum sub-rectangular, average measurements 0.02×0.05 mm.; lingula setose all over and armed distally with a pair of long hairs; average length 0.03 mm.

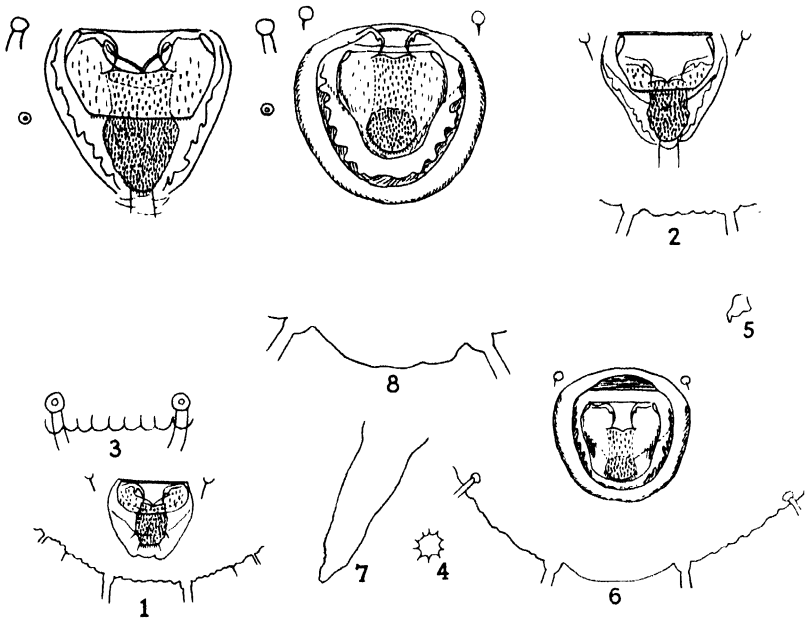


FIG. 1.—1–3, *A. lonicrae*, 1, vasiform orifice of 1st instar larva; 2, vasiform orifice of 2nd instar larva; 3, vasiform orifice of pupa; 4–8, *T. ericae*, 4, star-shaped structure on larva; 5, antenna of 3rd instar larva; 6, vasiform orifice of 3rd instar larva; 7, antenna of pupa; 8, vasiform orifice of pupa.

Pupa. Oval, ultimately becoming opaque and convex dorsally, colour variable between white, yellow or golden yellow; average measurements 1.05×0.74 mm. Margins dentate, surrounded by a thick rim of wax. Submarginal area transversely striated. Abdominal region marked usually with a row of concolorous tuberosities in the median line. Marginal spines three pairs—the anals prominent; dorsal spines 3–7 pairs, varying in size and distribution; ventrals may be absent. Legs degenerate; antennae, directed backwards and outwards, ending distally in a minute process, length about 0.07 mm. Vasiform orifice subcordate, average measurement 0.07×0.07 mm., inner lateral margins roughened, caudal margin slightly notched; operculum sub-rectangular, the caudal margin minutely hairy; average measurements 0.03×0.05 mm.; lingula cylindrical, thick and spatulate, setose all over, armed distally with a pair of long hairs; average length 0.05 mm., about $\frac{2}{3}$ exposed beyond the operculum (fig. 1).

*Adult*²—*Female.* Yellow, spotted with grey; head dark, mesothorax with one median,

² The descriptions, with the exception of the colour, apply both to grey and yellow individuals.

and two lateral spots, metathorax with a pair of median pear-shaped dark brown spots. Abdomen yellow, with a few faint (sometimes very conspicuous) transverse bands of brown, margins of the wax plates dark; last abdominal segment dark both dorsally and ventrally—leaving a circular yellow patch on the ventral side. Wings with one cloud distally, margins beset with a series of minute spines (fig. 2). Eyes divided, the halves separated by a streak, lower facets relatively larger and arranged in hexagons (fig. 2). Antennae darker distally, seven segmented (fig. 2), the 3rd the longest and provided with 3 sensoria distally, IV cylindrical, the smallest; V club-shaped with one sensorium near the tip; VI cylindrical, elongated; VII tapering abruptly, provided with a sensorium and a spine apically;

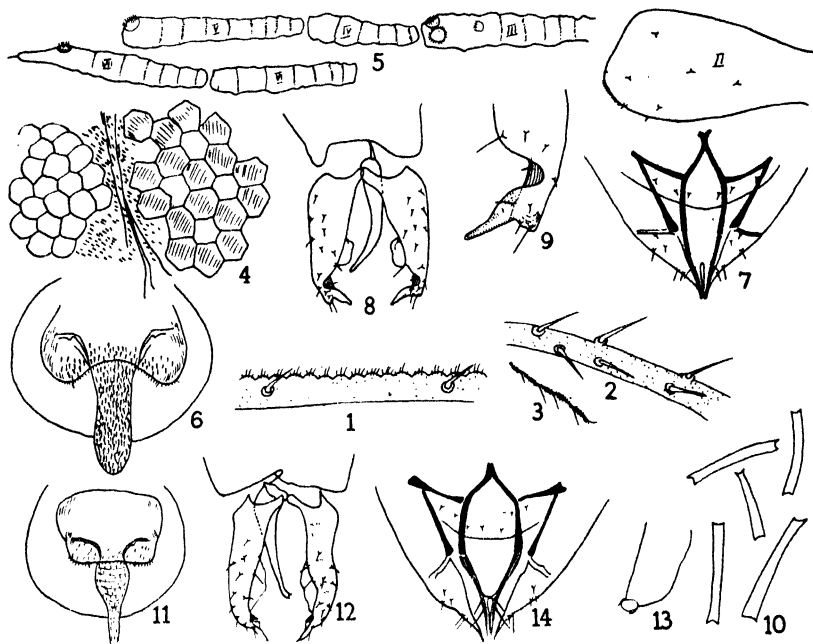


FIG. 2.—1-9, *A. lonicerae*, 1, costal margin of fore-wing; 2, costal margin of hind-wing; 3, lower margin of hind-wing; 4, eye of adult; 5, antenna of adult female; 6, vasisform orifice of adult female; 7, genitalia of female; 8, genitalia of male; 9, tip of a paramere; 10-14, *S. phillyreae*, 10, wax tubes on pupa; 11, vasisform orifice of adult female; 12, genitalia of male; 13, tip of aedeagus; 14, genitalia of female.

segments III-VII imbricate, measurements variable even within individuals. Vasisform orifice almost sub-circular, measuring, on an average, 0.07×0.08 mm. (fig. 2); operculum broad and sub-rectangular, caudal margin deeply notched, and hairy, average measurements 0.04×0.06 mm.; lingula long and stout, rounded at the tip, setose all over, almost entirely exposed, average length 0.04 mm. Genitalia as usual in the family, basal region of the inner valves provided with a single pair of prominent hairs (fig. 2).

Male. Colour may be lighter than the female. Vasisform orifice and the antennae correspond with those of the other sex, aedeagus curved, but not angulate. Parameres broader at the base, distally the inner margin is tucked into a notch forming a characteristic claw-shaped end with a basal heel armed with a pair of prominent bristles (fig. 2); length about 0.14 mm.

Aleyrodes carpini Koch 1857 and *A. rubicola* Douglas 1891.

These species have been regarded as distinct since they were first described, although no serious attempts have been made to examine them critically.

A. carpini was recorded originally by Koch (1857) from *Carpinus betulus* (hornbeam), and the colour of the adult was described. This, however, did not appeal to Frauenfeld (1867). Signoret (1868) simply copied the original description, but Douglas (1895) redescribed the adult and supplemented the description by a brief account of the pupa.

As a result of the extremely poor knowledge of this species, Quaintance and Baker (1914) could not even assign it a generic position in their classification of the ALEYRODIDAE. Harrison (1920) first introduced it under *Aleyrodes*, but soon after placed it under the genus *Asterochiton*. Marriner (1931), supported by Laing, disagreed with this change in generic position.

A. rubicola, on the other hand, was described by Douglas (1891), who recorded it from blackberry. His first attempt to distinguish it from *A. carpini* was based on its association with a different food-plant. He stated, "It is far more in agreement with *A. carpini* Koch; that species, however, has hitherto been believed to be special to hornbeam. The present form, I believe, is distinct; I name it provisionally *A. rubicola*." Towards the end of the same year he confirmed his views and described briefly the various stages, giving a few sketches. In the absence of any description of the immature stages of *A. carpini*, of which Douglas had no personal knowledge at that time, he anticipated that corresponding differential structures may exist even in their larvae. Since then the species has not been studied critically, and Quaintance and Baker (1914) classified it wrongly.

Presumably, therefore, these species were kept distinct on two characters: (1) the association of the forms with different food-plants, (2) on the relative proportion of the antennae which were stated to differ from the original diagram by Koch.

Present Observations.

The description of the various stages given under *A. carpini* practically agree with those found on blackberry. Observations in nature, as well as in the laboratory, have yielded conclusive data for regarding them as identical. This conclusion is based on the study of the following aspects:—

1. *Morphology.*

- i. Lack of significance of the previously supposed differences.
- ii. Special reference to the structure of the vasiform orifice in all the stages.
- iii. Study of the genitalia of the adult.

2. *Biology.*

- i. Appearance of the adults and their sex ratio.
- ii. Life-history and breeding tests.
- iii. Cross inoculations.

1. (i) The sketches of antennae given by Koch under various species of *Aleyrodes* are almost all open to criticism and are practically without exactitude. Douglas also submitted an equally inaccurate diagram with his description of the species *A. rubicola*. The antennal segment VII is shown quite blunt and without a terminal spine, also segment IV has been represented as almost a square. This is contrary to the actual structure (fig. 5). Similar inaccuracy

is noticed in his drawing of the "mature larva or pupa" of *A. rubicola*, in which the thoracic tracheal folds are represented but the caudal one is not shown. At the same time, no reference to either of these is given in the text, although these structures are very prominent in the pupa (figs. 3 and 4).

2. *Variations in spines.* Douglas, in his description of the "immature larva," stated "the six dorsal hairs are specially characteristic," but this is not a permanent character, as individual variations have been noticed in specimens collected in the field and bred in the laboratory.

The following arrangements have been met with in *rubicola* :—

Nymph 1st instar.

- i. Marginals, 17 pairs as described under *A. carpini*.
- ii. Dorsals, 3 pairs, antennal, abdominal and vasiformal, generally very minute, and occasionally more than three pairs may also be present.
- iii. Ventrals, 1-3 pairs, near the orifice but considerable variations in size have been noticed in these.

Nymphs 2nd and 3rd instar.

Marginal spines are reduced to 3 pairs only, cephalic minute, caudo-laterals small and the anals developed as referred to under *A. carpini*.

On the other hand, considerable variations have been noticed in the dorsal and ventral spines in these instars and the various combinations are represented in Table IV.

A remarkable feature observed in this connection was the development of spines even on *A. carpini* type when bred on blackberry. On the contrary, the spines almost disappeared even on *A. rubicola* when these were bred on horn-beam. It may therefore be concluded that probably the food-plant plays an important part in bringing about these variations. Deshpande (1933) discovered a corresponding effect of the food-plant on the pupae of *A. loniceræ*. Takahashi (1933) also noticed that the pupae of *Acanthaleyrodes callicarpæ* Tak. and other species which were associated with hairy leaves developed dorsal spines.

Douglas, in his account of the "mature larva" of *A. rubicola*, mentions certain notches or constrictions of the margin. My investigations have shown that these irregularities in the pupal form are only associated with the presence of hairs or some roughening on the leaf. In the absence of any of these, the larvae present quite a regular outline. Further, these folds have not been observed to show any definite arrangement and may vary in number and position with individuals.

- ii. *Structure of the vasiform orifice.* This character seems to be very significant in showing the morphological identity of the forms as is evident from fig. 3.

The shape of the component parts of this organ in 1st instar nymphs of both so-called species is quite identical and the characteristic latero-posterior notches from the operculum are quite significant. The average measurements in *A. rubicola* type compare almost exactly with those of *A. carpini*.

In the 2nd instar, as well, the shape and structure are identical in both forms. Average measurements in *A. rubicola* type are almost equal to that in *A. carpini*. The inner lateral margins of the vasiform orifice are similarly thickened.

In the 3rd and pupal instars, the average measurements are alike and the operculum and lingula in both are brownish, the inner lateral thickenings in the vasiform orifice even extend medially and the floor is also thickened.

This organ presents quite identical characters even in the adults. The free margin of the operculum is slightly notched and the lingula is short and thick with a characteristic rounded tip.

TABLE IV.

Types of distribution and development of dorsal and ventral spines met with in the nymphs of *A. carpini* on *Rubus* sp. (*rubicola* type).

1st instar

No.	Dorsal											Ventral			
	Antennal Pairs				Abdominal Pairs						Vasiformal Pairs		Vasiformal Pairs		
	I		II		Anterior				Posterior	Anterior	Lateral	Anterior	Lateral	Posterior	
					I		II								
	1	2	1	2	1	2	1	2							
1	---	---	+	+	---	---	++	+	++	---	---	+	+	++	
2	---	---	○	○	---	---	○	○	○	---	---	○	○	○	
3	---	---	○	○	---	---	○	○	○	---	---	○	○	○	

2nd instar

No.	Dorsal						Ventral					
	Antennal		Abdominal		Vasiformal		Antennal		Abdominal		Vasiformal	
	1	2	1	2	1	2	1	2	1	2	1	2
1*	---	---	---	---	---	---	○	○	○	○	○	○
2*	---	---	---	---	---	---	○	○	○	○	○	○
3	++	++	++	++	+	+	○	○	○	○	○	○
4	++	++	++	++	+	+	○	○	○	○	○	○
5	++	++	++	++	++	++	○	○	○	○	○	○
6	++	++	++	++	++	++	○	○	○	○	○	○
7	++	++	++	++	++	++	○	○	○	○	○	○
8	++	++	++	++	++	++	○	○	○	○	○	○
9†	++	++	++	++	++	++	○	○	○	○	○	○
10	+	+	++	++	++	++	○	○	○	○	○	○
11	+	+	++	++	++	++	○	○	○	○	○	○
12	+	++	++	++	++	++	○	○	○	○	○	○
13	+	+	+	+	+	+	○	○	○	○	○	○

— Minute. --- Very minute. + Developed. ++ Well developed. ○ Absent.

* Bred on hornbeam.

† Two pairs of ventrals, one anterior and the other posterior to vasiform orifice, both developed.

iii. The genitalia in both male and female are identical and the description given under *A. carpini* fits nicely to the other form as well.

The structure of the eyes and antennae of the adults gave further evidence in support of their identity. The eyes are identical in structure, since the two halves in both the forms are connected by a bridge of one facet only (fig. 5). Correspondingly, the antennal segments also are of almost identical shape. Segment IV, however, differs strikingly in shape from that shown by Douglas.

In view of the uniformity in these morphological characters both in the larvae and the adult, it is recognised that both forms are indistinguishable from each other.

2. Biology.

i. *Appearance of the adults and their sex ratio.* The earliest adult, in nature, was collected on 20.v.1937 on hornbeam, after which the number increased in the field. Their distribution, however, was restricted to certain localities both on hornbeams and blackberries. Observations during May and June showed that the females considerably outnumber the males, and, on an average, were estimated at about 75% of the total catch. The adults, as a rule, are short-lived and their numbers show a pronounced decrease towards the end of June, when only a few stray specimens may be collected in the field. During 1937 adults were not observed in nature from July onwards.

ii. *Life-history.* Eggs are laid on the underside of the leaves, but may be noticed casually on the upperside as well, both in nature and in captivity. In nature they are laid scattered, and seldom more than three nymphs have been observed on a leaf. In captivity, however, they may be laid close together but never in circles. The eggs are dusted very lightly with a waxy powder but it does not make the surrounding area in any way conspicuous. Females are very active and take to flight at the slightest provocation.

The species is single brooded, oviposition in nature commences actively towards the end of May. The duration of the egg stage, during 1937, extended from 30 to 36 days during June and July. The duration of the 1st instar lasted from 15 to 27 days and of the 2nd instar from 14 to 20 days (Table V). The earliest pupa was noticed on 12.viii., but this was in no way a representative case because this brood was from eggs laid in the first week of May (the pupae collected from the fields were kept in a hot-house from which the adults emerged and laid eggs in captivity much before they were obtained in nature). In nature, however, only the 3rd instar nymphs were met with during that part of the year, and pupae from the respective food-plants were collected in early September.

Overwintering takes place in the pupal stage. The leaves with the pupae remain hanging on the trees or bushes for a long time or may fall to the ground, where the insect passes the winter. From pupae bred in the laboratory and those collected in the field, when kept indoors from October 1937 onwards, the adults commenced emerging from the first week of March, 1938.

Observations during April and May show that some of these pupae are parasitised and the extent of parasitism may be as high as 40%.

iii. *Cross inoculation.* Cross inoculations of the so-called species *A. carpini* and *A. rubicola* were tried and development noted. Adults were obtained in the laboratory from pupae collected separately from hornbeam and wild blackberry. They were then bred on their respective alternative food-plants. The general behaviour of all the stages in both cases was similar and the insects thrived well and developed quite normally on the alternative plants (Table V).

Alternative host-plants. The species is not confined to hornbeam and blackberry. Adults have been collected from raspberry, oak and hazel as well, but the examples may have been casual visitors. During September and October, 1937, however, numerous pupae of this species were collected on hazel³ at Bricketwood. This provides considerable evidence to indicate that the species thrives quite well on this food-plant in nature. The adults from these pupae emerged in March, 1938, in the laboratory, approximately at the same time as those emerged from the pupae on hornbeam.

³ Probably this coincidence was responsible for placing *A. carpini* and *A. avellanae* under the genus *Asterochiton* as proposed by Harrison.

TABLE V.

Life-cycle and cross inoculations of *A. carpini*, on alternative host-plants, 1937-1938.

Adults collected from	Life-cycle completed on	Date of introducing the adults	Earliest date of hatching of eggs	Earliest date of 1st moult	Earliest date of 2nd moult	Earliest date of emergence of adults	Shortest duration of life-cycle in days
<i>Rubus fruticosus</i>	<i>Carpinus betulus</i> ¹	1937	1937	1937	1937	1938	
<i>Carpinus betulus</i>	<i>Rubus fruticosus</i> ²	2.v.	7.vi.	28.vi.	12.vii.	6.iii.	308
<i>Carpinus betulus</i>	<i>Rubus fruticosus</i>	23.v.	28.vi.	—	—	8.iii.	289
<i>Carpinus betulus</i>	<i>Rubus fruticosus</i>	30.v.	3.vii.	21.vii.	10.viii.	10.iii.	284
<i>Carpinus betulus</i>	<i>Carpinus betulus</i>	1.vi.	2.vii.	18.vii.	—	9.iii.	281
<i>Rubus fruticosus</i>	<i>Carpinus betulus</i>	2.vi.	3.vii.	21.vii.	9.viii.	6.iii.	277
<i>Rubus fruticosus</i>	<i>Carpinus betulus</i>	5.vi.	7.vii.	23.vii.	—	6.iii.	274
<i>Carpinus betulus</i>	<i>Carpinus betulus</i>	9.vi. ³	10.vii.	26.vii.	10.viii.	6.iii.	270
<i>Rubus fruticosus</i>	<i>Rubus fruticosus</i>	11.vi. ¹	12.vii.	—	—	8.iii.	270
<i>Rubus fruticosus</i>	<i>Carpinus betulus</i>	11.vi.	10.vii.	26.vii.	9.viii.	3.iii.	265
<i>Carpinus betulus</i>	<i>Carpinus betulus</i>	11.vi. ¹	13.vii.	28.vii.	—	6.iii.	268
<i>Rubus fruticosus</i>	<i>Rubus fruticosus</i>	15.vi. ³	—	—	—	8.iii.	266
<i>Carpinus betulus</i>	<i>Rubus fruticosus</i>	15.vi. ¹	16.vii.	—	—	8.iii.	266
Pupae on <i>Corylus avellana</i> ²	—	—	—	—	—	3.iii.	—

¹ Hornbeam.² Blackberry.³ Numerous pupae of the *carpini* species were collected from hazel at Bricketwood on 5.x.1937. The adults from them emerged in March, 1938.

The pupae from different experiments given above were kept in the laboratory, hence the date of emergence is comparatively earlier than in nature.

*Description of the stages of A. carpini*⁴ Koch (figs. 3, 4, 5).

Egg. Oblong, apical end narrower, pedunculate, chorion smooth: light yellow when fresh, subsequently turns to dark brown; average measurements 0.24×0.10 mm., stalk about 0.03 mm.

Nymph 1st instar. Elliptical; light yellow but turns to brownish later on; margins surrounded by a waxy fringe, average measurements 0.23×0.20 mm. Marginal spines 17 pairs; dorsal spines three pairs, minute—antennal, abdominal, and vasiformal. Ventral spines—vasiformal. Antennae developed, three segmented, the 3rd the longest and provided with two spines (fig. 3), average length 0.08 mm. Eyes divided; legs functional. Vasiform orifice 0.04×0.03 mm., inner lateral margins not thickened; operculum nearly semicircular with a latero-posterior notch at the caudal margin, average measurements 0.02×0.03 mm.; lingula relatively expanded towards the posterior half, a few prominent hairs laterally at the distal end; length 0.02 mm. and more than half exposed beyond the operculum (fig. 3).

Nymph 2nd instar. Oval, yellowish-green; margin crenulate with a narrow waxy fringe all round, average measurements 0.46×0.32 mm. Marginal spines three pairs, cephalic, and caudo-lateral, minute, anals prominent; dorsal spines three pairs, minute; ventrals 1-2 pairs; submarginals as 4 or 5 minute hooks, running posterior to the mycetoma. Eyes entire; legs degenerate; antennae atrophied, directed backwards (fig. 3) and, on an average, 0.02 mm. long. Vasiform orifice pyriform, average measurements 0.05×0.04 mm., inner lateral margins roughened by thickening; operculum almost semicircular, caudal margin hairy, average measurements 0.02×0.04 mm.; lingula long and thick, spatulate, armed distally with a pair of prominent hairs; average length 0.03 mm. and more than half exposed beyond the operculum (fig. 3).

Nymph 3rd instar. Shape as in previous instars, broadly flattened dorso-ventrally, margins dentate; yellow-green, almost concolorous with the leaf; average measurements 0.78×0.57 mm.; marginal waxy fringe very narrow, thoracic tracheal folds evident. Spines as in the preceding instar. Eyes entire or divided; legs degenerate; antennae atrophied, directed inwards and ending in a hook (fig. 3), length about 0.03 mm. Body of

⁴ For generic position see p. 585.

the developing insect marked by an *oval*; abdominal segmentation obvious and marked by transverse partitions, each of which bears minute circular tuberosities towards its extremities. Vasiform orifice pyriform, with a furrow extending to the caudal margin; average measurements 0.08×0.06 mm., inner lateral margins and the floor of the orifice roughened by thickenings; operculum and lingula brownish in colour; operculum almost semicircular, average measurements 0.03×0.05 mm.; lingula as in the previous instar, average length 0.06 mm. (fig. 3).

Pupa. Elliptical, greenish-yellow when newly transformed, whitish-opaque later, broad, slightly convex dorsally; margin crenulate and fixed to the leaf; submarginal area wide

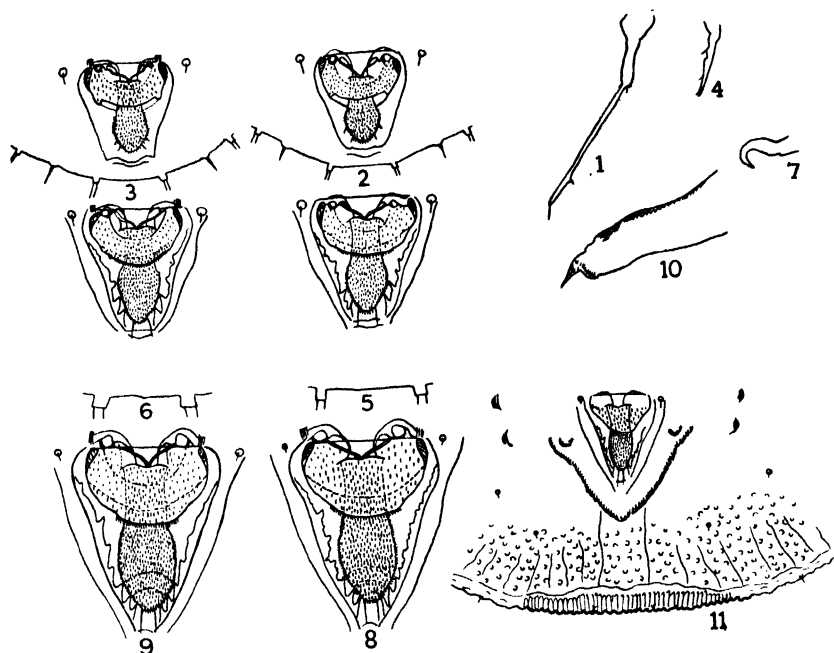


FIG. 3.—*A. carpini*, 1, antenna of 1st instar larva; 2, vasiform orifice of 1st instar larva from hornbeam; 3, vasiform orifice of 1st instar larva from blackberry; 4, antenna of 2nd instar larva; 5, vasiform orifice of 2nd instar larva from hornbeam; 6, vasiform orifice of 2nd instar larva from blackberry; 7, antenna of 3rd instar larva; 8, vasiform orifice of 3rd instar larva from hornbeam; 9, vasiform orifice of 3rd instar larva from blackberry; 10, antenna of pupa; 11, anal margin and vasiform orifice of pupa.

with concolorous transverse striations, average measurements 1.23×0.92 mm. Outline of the developing insect differentiated by an *oval* formed of whitish warts. Three tracheal folds—two thoracic and one anal—each ending at the margin of the pupa in a comb of teeth with a brittle, white frill of wax protruding beyond the margin. Marginal spines extremely minute, caudo-laterals prominent; dorsal spines two pairs, minute; ventrals 1 or 2 pairs and submarginals 2–4 pairs. Eyes entire, antennae directed backward and outward, and ending in a narrow process (fig. 3); about 0.07 mm. long; legs curved, degenerate. Vasiform orifice elongated, triangular, longer than broad; average measurements 0.10×0.07 mm., inner lateral margins and the floor of the orifice thickened; operculum and lingula brownish; operculum semicircular, average measurements 0.04×0.06 mm.; lingula stout and spatulate and armed distally with a pair of prominent hairs;

average length 0.07 mm. and rather more than half exposed beyond the operculum (figs. 3 & 4).

Adult. (Male.) Yellow or light orange in colour, body without any sculpture, about 1.1 mm. in length, wings white, marginal veins bright yellow; tip of rostrum brown. Eyes constricted, both the halves united by a bridge of a single facet (fig. 5). Antennae of seven segments—I sub-globose, II pyriform, beset with a few minute spines, III longest of all and beset with three well-developed sensoria distally, IV sub-cylindrical, the smallest, V almost clavate with one sensorium at the distal end, VI elongate, VII cylindrical, tapering distally and provided with a sensorium and ending in a spine; segments from III

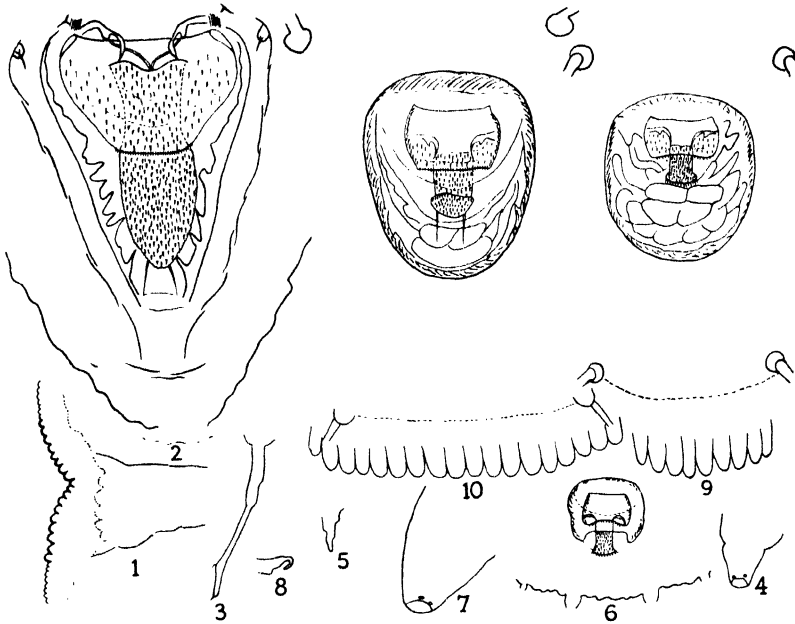


FIG. 4.—1-2, *A. carpini*, 1, thoracic tracheal fold of pupa; 2, vasiform orifice of pupa; 3-9, *S. phyllirae*, 3, antenna of 1st instar larva; 4, leg of 2nd instar larva; 5, antenna of 2nd instar larva; 6, vasiform orifice of 2nd instar larva; 7, leg of 3rd instar larva; 8, antenna of 3rd instar larva; 9, vasiform orifice of pupa; 10, *S. immaculata*, vasiform orifice of pupa.

to VII imbricate. Vasiform orifice broader than long; operculum rectangular; average measurements 0.03×0.05 mm.; caudal margin slightly arched and hairy; lingula short and thick, tip rounded and armed with minute hairs; length about 0.03 mm. (fig. 5); parameres medium in size, length about 0.14 mm., aedeagus long and almost straight, length 0.12 mm. (fig. 5).

Female. Concolorous with the male, may be slightly bigger. Antennae a little longer than in the male (see Table VI). Abdomen relatively broader at the base. Vasiform orifice as in the male. Genitalia as usual in the family; each dorsal valve provided with a pair of prominent hairs near the basal region of the inner valves (fig. 5). (For measurements of antennal segments see Table VI.)

Asterobemisla gen. n.

Prior to the classification of ALEYRODIDAE by Quaintance and Baker (1913-14) practically all the European species of this family were listed under the
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genus *Aleyrodes*. Quaintance and Baker did not assign *A. carpini* to any genus but included *A. rubicola* under *Aleyrodes*. Marriner and Laing (1931) were satisfied with its original position under *Aleyrodes*, but Harrison (1920 and 1931), on the other hand, shifted *A. carpini* to *Asterochiton* but retained *A. rubicola* under *Aleyrodes*. Somehow the identity of these forms seems to have been overlooked, and this unfortunately resulted in a different systematic position even with regard to the genera.

The genus *Asterochiton* was founded by Maskell (1879) with his species *lecanioides* and was placed in the COCCIDAE. Quaintance and Baker (1914), however, regarded Maskell's type as an example of *vaporariorum*. Baker and

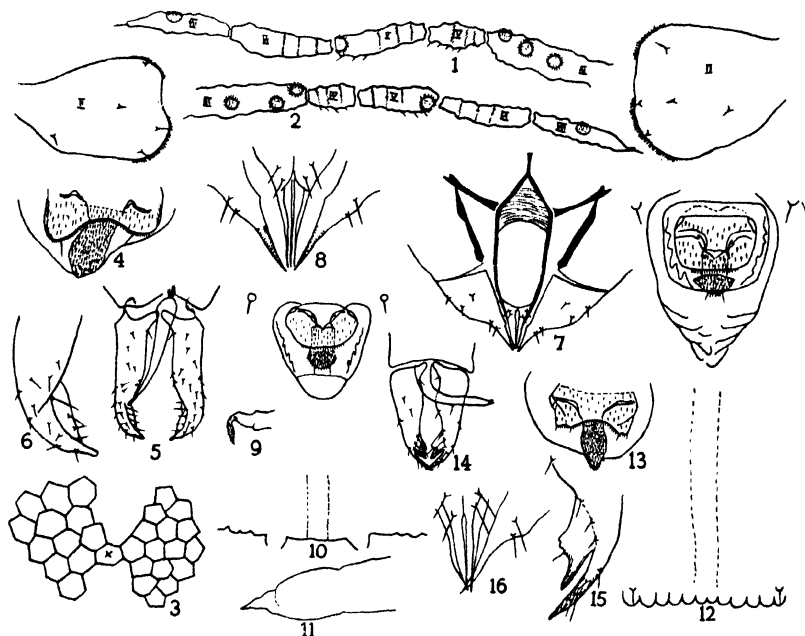


FIG. 5.—1-8, *A. carpini*, 1, antenna of female; 2, antenna of male; 3, eye of adult; 4, vasiform orifice of adult; 5, genitalia of male; 6, tip of a paramere; 7, genitalia of female; 8, ovipositor of female; 9-16, *P. quercus*, 9, antenna of 3rd instar larva; 10, vasiform orifice of 3rd instar larva; 11, antenna of pupa; 12, vasiform orifice of pupa; 13, vasiform orifice of adult female; 14, genitalia of male; 15, tip of a paramere; 16, ovipositor of female.

Moles (1921) maintained the genus *Asterochiton* but specified different characters. The new generic characters were summarised by Singh (1931). Comparing the characters given under *Asterochiton* with those given under *Dialeurodoides* by Quaintance and Baker (1914), it appears that the former has simply replaced the latter.

It may be pointed out that the structure of the pupa in *A. carpini* and the long triangular nature of the vasiform orifice, do not bring this species in any way near to the old genus *Asterochiton* or the one described later by Baker and Moles. Morphologically the structure of the vasiform orifice in *A. carpini* is almost similar to that described under *Bemisia*, but it differs in having no caudal furrow; the presence of the tracheal folds ending in a comb of teeth,

the oval formed by a series of warts concolorous with the body and the structure of the vasiform orifice, form a characteristic structural combination, which brings it between the above genera. The new genus *Asterobemisia*, therefore, is proposed, with *Aleyrodes carpini* Koch as the type species.

TABLE VI.

Measurements of antennae in the male and female of *Asterobemisia carpini*.

Sex	Average measurements of antennal segments							Total length of the antenna
	I	II	III	IV	V	VI	VII	
♂♂	0.03 0.03	0.06 0.07	0.13 0.15	0.02 0.02	0.03 0.04	0.03 0.04	0.04 0.05	0.34 mm. 0.40 mm.

Aleyrodes proletella Linn. 1758 and *A. brassicae* Walker 1852.

The species under discussion was the first to attract the attention of naturalists. Réaumur (1736) described a species with two spots on the elytra and included it in the Lepidoptera. Linnaeus (1758) named the species *proletella* and mentioned *Brassica* and *Chelidonium* as its food-plants. Roemer (1789) briefly described the species *chelidonio* but Latreille (1796) diagnosed its hemipterous characters and described the species again. Burmeister (1835) published a slightly better account of the insect giving, however, only superficial characters which apply to the whole group; the diagram of the adult shows a forked vein in the wing. Westwood (1840) recorded *A. proletella* infesting cabbages, *Chelidonium* and oaks. Ruricola (1846) described *A. proletella* but doubtfully regarded the antennae as five segmented. His observations, "infesting cabbages, called also by Latreille *A. chelidonio* from its living upon the great Celandine (*Chelidonium majus*)," yield evidence that both forms were considered identical. Walker (1852) regarded *A. brassicae* as a variety of *A. proletella*. Koch (1857) described some of the superficial characters of the adult of *A. brassicae*. Frauenfeld (1867) reviewed the previous accounts of *A. brassicae* by Walker and Koch and considered it distinct from *A. proletella*. Signoret (1868), on the other hand, presented a much fuller account of the species, and described the colour of the adult *A. proletella* and added a detailed account of its immature stages. His description of *A. brassicae* was equally complete and some supposed differentiating characters in the 1st instar larvae were given. Douglas (1895) considered the two forms as distinct species, but his observations were based mostly on preserved material. He supported Signoret with respect to the differentiating characters in the 1st instar larvae without examining that stage. Tullgren (1907) was the first to state the identity of the forms on morphological characters. He was convinced that the differences pointed out by Signoret and Douglas were not genuine and that the forms were identical. Laing (1921), in a note on *A. proletella* (L.), stated "this species is not confined to the genus *Brassica*, it also is to be found on *Chelidonium majus*. It is generally stated that another species (*A. brassicae* Walker) is also found on cabbage, but I have not yet been able to satisfy myself as to whether this is really a distinct species." Marriner (1931), on the other hand, regarded each of these forms as distinct species. Deshpande (1933) gave a detailed account of *A. brassicae*, and maintained the individuality of the

respective species *A. proletella* and *A. brassicae*. On the contrary, Haupt (1935) considered both forms identical in their morphological characters, and Weber (1935) described the genitalia in *A. brassicae*.

*Present investigations and the evidence in favour of the identity of
A. proletella and A. brassicae.*

1. *Structural.*

The basic differential characters as suggested by Signoret and supported by Douglas were to be found in the larvae of the 1st instar. Signoret remarked that the larvae of *A. proletella* were oval and showed all round a border of fairly long hairs, in all 34-36, the four longest being at the extremity of the abdomen. In *A. brassicae*, on the other hand, the larva was stated to be more elongated and less rounded, with hairs only on the abdominal segments and with only two long hairs towards the extremity.

During the present investigation, an examination of over 100 1st instar larvae of *A. brassicae* has shown that 16 or 17 pairs of marginal spines are present all round the larva, including the thoracic as well as the cephalic regions. Further, two of the posterior pairs, with a small spine between them on each side, are long and prominent (fig. 6). These observations are in agreement with those of Deshpande (1933), who described the 1st instar larva of *A. brassicae* and stated: "There is a rimlike narrow margin from which 16 pairs of small hairs arise, of which one anterior and two posterior pairs are prominent and long." Hence, it may be concluded that the difference noted by Signoret in the distribution of their marginal hairs and the prominence of a single posterior pair of bristles in *A. brassicae*, was most probably the result of restricted observations or defective technique.

To verify the significance of the suggested difference in the shape of these nymphs, measurements of 43 individuals of both forms, bred in the laboratory, were taken for comparison. Two factors were taken into consideration: namely, the length of the individuals and the relative ratio of their length to breadth.

Considerable variations have been noticed in the individuals of both the forms, but there appeared no indication that their average measurements were in any way different, or that individuals from two different food-plants belonged to different populations.

Besides, the comparative study of the various stages yielded conclusive evidence that the various stages of *A. brassicae* are inseparable morphologically from those of the allied form on *Chelidonium majus*, hitherto known as *A. proletella*. The forms are, therefore, considered identical and may be called *A. proletella* Linn., this being the earlier name.

2. *Biological.*

a. *Cross inoculations.* Adults bred from their respective food-plants were transferred to alternative plants, where the respective males and females from different plants mated and laid eggs. The immature stages flourished without affecting the life-cycle (Table VII). It was observed that the shortest duration of the life-cycle on both plants varied from 42 to 48 days during April and May, and from 33 to 36 days during June to September.

b. *Comparative oviposition.* To determine any preference in the food-plants by the adults, relative oviposition was studied under laboratory conditions simultaneously on cabbage and *Chelidonium majus*. Cabbage seedlings were grown in pots which contained small shoots of Celandine and a known

number of newly emerged adults of *A. proletella*—10 or 12 females with half the number of males, was introduced for oviposition under lamp glasses. Two such experiments, each with two repetitions and with an exposure of four days, gave the following results :—

Host-plant	Leaf area, sq. cm.	No. of eggs laid	Eggs per sq. cm.
1. Cabbage	94.6	93	0.98
Celandine	235.6	40	0.17
2. Cabbage	94.3	85	0.90
Celandine	201.1	31	0.15

Eggs were laid readily on both the host-plants but in spite of the relatively greater leaf area of Celandine, the insect showed some preference for cabbage.

c. General remarks and food-plants. The species is found all the year round and overwinters in the pupal as well as in the adult stage. The eggs are laid in rings, although occasionally a few scattered ones were also noticed, and are dusted over by white waxy powder. The maximum number of eggs laid by a single female, in captivity, from 22.vi. to 1.ix., was 219 in the 70 days. The same female also gave the maximum duration of life of an adult during summer.

The species, however, is not confined to its recorded food-plants. During May, in nature, adults have been observed laying eggs on wild *Sonchus* sp., on which the immature stages develop. Besides, the adults have also been noticed taking shelter on strawberry plants during winter. Although the species is not known to breed on strawberries in nature, nevertheless attempts made in captivity were successful. In one case the life-cycle could not be completed but in the second case (Table VII) the cycle was completed successfully.

TABLE VII.

Life-cycle and cross inoculations of *A. proletella* on alternative hosts.

Adults bred from	Life-cycle completed on	Date of intro- ducing adults	Earliest date of the hatching of eggs	Earliest date of the emergence of adults	Shortest duration of life-cycle
<i>Brassica oleracea</i>	<i>Chelidonium majus</i>	1937 5.iv.	1937 19.iv.	1937 17.v.	42
<i>Brassica oleracea</i>	Strawberry (<i>Fragaria</i> sp.)	5.iv.	20.iv.	23.v.	48
<i>Brassica oleracea</i>	<i>Chelidonium majus</i>	8.iv.	23.iv.	26.v.	48
<i>Brassica oleracea</i>	<i>Brassica oleracea</i>	12.iv.	26.iv.	27.v.	45
<i>Chelidonium majus</i>	<i>Brassica oleracea</i>	21.vi.	—	25.vii.	34
<i>Chelidonium majus</i>	<i>Brassica oleracea</i>	*22.vi.	—	26.vii.	34
<i>Chelidonium majus</i>	<i>Brassica oleracea</i>	23.vi.	—	27.vii.	34
<i>Brassica oleracea</i>	<i>Chelidonium majus</i>	23.vi.	—	26.vii.	33
<i>Brassica oleracea</i>	<i>Chelidonium majus</i>	29.vi.	9.vii.	4.viii.	36
<i>Chelidonium majus</i>	<i>Brassica oleracea</i>	7.viii.	15.viii.	12.ix.	36

* Maximum number of 219 eggs was laid by a single female in 70 days.

Besides the genus *Brassica* and Celandine, this species was observed for the first time, on 27.ix.1937, breeding freely on Canterbury bells (*Campanula* sp.).

Description of the stages of A. proletella Linn. (fig. 6).

Deshpande (1933) incorporated the description of all the stages in his account of *A. brassicae*. It is not justifiable to repeat the descriptions in full, but it is desirable to supplement them with a view to pointing out differences or filling up gaps.

Nymph 1st instar. According to Deshpande the larva remains active for three days. This statement, however, does not agree with my observations. Forty-five newly hatched nymphs were examined for this purpose and many more connected with other observations, but in most of the cases they were fixed practically on the day after hatching. The prolonged activity noticed

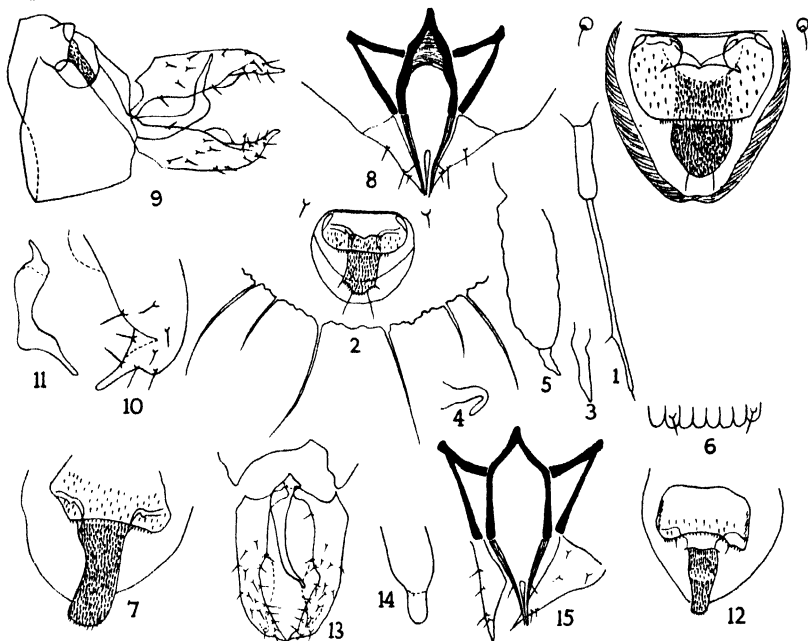


FIG. 6.—1–11, *A. proletella*, 1, antenna of 1st instar larva; 2, vasiform orifice of 1st instar larva; 3, antenna of 2nd instar larva; 4, antenna of 3rd instar larva; 5, antenna of pupa; 6, vasiform orifice of pupa; 7, vasiform orifice of adult; 8, genitalia of female; 9, last abdominal segment and genitalia of male; 10, tip of a paramere; 11, aedeagus of male; 12–15, *S. immaculata*, 12, vasiform orifice of adult; 13, genitalia of male; 14, tip of aedeagus; 15, genitalia of female.

by Deshpande might have been an extraordinary feature restricted to certain food conditions. Eyes are quite distinct and may be entire or divided. The antennae (fig. 6) are long and measure about 0.11 mm. The dorsum is not devoid of spines, but possesses three pairs of minute spines—antennal, thoracic and vasiformal. Besides, two or occasionally three pairs of ventral spines are also visible. Vasiform orifice sub-circular, slightly broader than long (fig. 6), average measurements 0.03×0.04 mm.; operculum sub-rectangular, faintly hairy on its caudal margin, average measurements 0.01×0.03 mm.; lingula long, cylindrical, setose all over, distal end with a pair of long hairs and a relatively shorter one on either side, average length about 0.02 mm. and about $\frac{3}{4}$ exposed beyond the operculum.

Nymph 2nd instar. Marginal spines 2-3 pairs, dorsal spines also 2-3 pairs. Deshpande missed these but mentioned a pair cephalad to vasiform orifice late in the pupa, which gives the impression that probably they appear in that stage for the first time. On the contrary, that particular pair is present in all nymphal stages. Eyes small, reddish and generally divided. The antennae, not mentioned by Deshpande, are present but reduced, directed backwards (fig. 6), average length 0.02 mm. Vasiform orifice almost subcordate, average measurements 0.04×0.05 mm.; operculum sub-rectangular, 0.02×0.04 mm.; lingula cylindrical, a little spatulate and with a pair of long prominent hairs at the tip, average length 0.02 mm. and about $\frac{2}{3}$ exposed beyond the operculum.

Nymph 3rd instar. Margin dentate, spines as in the previous instar. Antennae atrophied, directed inward and hooked, average length 0.03 mm. (fig. 6). Eyes entire. Deshpande's remark "eyes appear as small red spots" gives an impression that probably they appear in this stage for the first time, but they are quite conspicuous in all the instars. Vasiform orifice subcordate, inner lateral margins thickened, average measurements 0.05×0.06 mm.; operculum sub-rectangular, measurements 0.03×0.05 mm.; lingula thick and cylindrical, average length 0.03 mm., about $\frac{2}{3}$ exposed beyond the operculum.

Pupa. Marginal spines 1-3 pairs, the anals always present but invariably minute. This is not in agreement with Deshpande's observations, who mentions them as a pair of long spines. The antennae directed backwards and outwards and ending in a small process (fig. 6). Vasiform orifice subcordate with the inner lateral and caudal margins uniformly thickened, measurements 0.07×0.07 mm. (fig. 6); operculum about 0.03×0.05 mm.; lingula as in the previous instar, average length 0.04 mm.

Adults. Deshpande's description of the adults is far more detailed than that of the immature stages. The male and female genitalia, however, require some comment because his figures appear too diagrammatic. In the female, the basal sclerites are shown extending to the margin of the body, evidently much longer than they are in fact. As a rule, these flat basal portions never extend beyond the junctions where they meet the lateral ones. Also, the dorsal valves are shown running in continuation with the inner chitinous press (terms after Weber, 1935), indicating, thereby, no structural difference. In well-prepared specimens these valves are clearly marked out and are distinct from the press mentioned above (fig. 6).

The diagrams and interpretation of the male genitalia do not agree with my observations. Deshpande remarked that the parameres (claspers) are "deeply forked" but this is not found to be the case (fig. 6). The aedeagus, which is represented as almost straight, is found to be angularly curved, more or less boot-shaped in outline (fig. 6).

This feature is quite characteristic of the species and gives strong evidence in support of the identity of the two forms *A. brassicae* and *A. prolella*. Tullgren (1907) also explained it as "Der penis stark winkelig aufgebogen."

Aleyrodes quercus Signoret 1868 and *A. avellanae* Signoret 1868.

Both these species were originally described by Signoret (1868), *quercus* on p. 384 and *avellanae* on p. 385. Following is a brief translation of the description of the adult and the larva of *A. quercus* :—

"*Adult.* Yellow-brown on the body and pale yellow on the abdomen; spotted with blackish. Head globular; vertex black, front brownish-black, cheeks yellow. Antennae greyish-yellow, eyes strongly divided. The pro-, meso- and metathorax blackish, sutures

pale. Black spots on median chest. Abdomen strongly pedunculate, yellow and black at the tip. Legs blackish, tibiae lighter, generally yellowish. Claws of the sexual organs of male blackish and developed. Elytra with a large blackish spot at the extremity of the median nerve. Sometimes, as in other species, pale individuals without spots are met with.

"*Larva*. Very remarkable. All that is outside the actual body of the insect is regularly punctate, the punctations radiating from the body and get larger towards the circumference. Each abdominal segment shows on each side a tubercle in the youngest larvae . . ."

The same author gave an account of the larva under *A. avellanae* and a copy of a translation by Douglas is quoted here :—

"But the larva is easy to distinguish, since it has, like that from the oak, some cavities in the form of excoriations on the median line of the abdomen; the sides, or expansion around the body, are much broader, more transparent, more foliculated, very wrinkled, and at the point of distinction of this expansion of the body properly so-called, there are also the same kind of excoriations on each segment, eight on each side and some of them also near the cephalic portion. On each side of the median line, on the first and second abdominal segment, is a blackish spot; the extremity of the abdomen or anus elongate and brownish . . ."

Douglas (1894) described, very briefly, the adults of *A. avellanae* as entirely "gamboge-yellow; eyes black, not divided; wings white, transparent, without a dark spot, marginal nerve yellow." Quaintance and Baker (1914) assigned *A. quercus* to the genus *Aleyrodes* but did not propose any generic position for *A. avellanae*. Since then both species have been totally ignored except for a few references in literature. Harrison (1920 and 1931) considered the generic position of both forms and placed *A. avellanae* in the genus *Asterochiton*, but retained *A. quercus* under *Aleyrodes*. Deshpande (1933) mentioned briefly the eye and the reproductive system of *A. quercus*. Haupt (1935) stated a few characters of the pupa and the adult of *A. quercus*; but his account appears to be a translation of Signoret's description.

It may be concluded that Signoret proposed the differentiating characters in the larvae of *A. avellanae*, which were stated to have a broader margin than in *A. quercus* and a pair of blackish spots on the 1st and 2nd abdominal segments. Douglas, on the other hand, considered the food-plant as a diagnostic feature. He described the adult of *A. avellanae* without any black markings.

Present Investigation.

The species under discussion needs comments in respect to the description of the stages as well as its systematic position.

1. Adult (*A. quercus* type). In 1932, Dr. C. B. Williams received a few adults collected from oak plants at Wye, Kent, and in 1935 discovered the species in small numbers at Batford, Herts. This material was kindly given to me for examination. The adults were entirely yellow and without any brown markings on the body. The parameres in the male were relatively small in size and concolorous with the abdomen. On 1.vi.1937 I collected one male specimen from oaks at Batford, Herts, which possessed similar characters to those described above. The genitalia was identical to that observed in the specimens sent from Wye during 1932. During October, 1937, some pupae were collected from oak and hazel in Bricketwood, Herts, and kept in a hot-house. A few adults emerged from these pupae in March and April, 1938. Hence the convincing information regarding colour and structure was obtained from the adults which emerged in the laboratory. These adults

were uniformly yellow and without any grey markings on the body. The genitalia of the males was exactly similar to that found in the previous examples.

It is evident from these facts that Signoret's adults of *A. quercus* were not specimens of this species. As already suggested, it appears probable that the adults of *A. lonicerae* were mistaken for this species when collected from oak, where they generally take shelter during autumn.

The adults that emerged from the pupae on hazel (*A. avellanae* type) almost simultaneously with those from oak, were also pure yellow and resembled in all characters those from oak. It is, therefore, possible that the specimens described by Douglas as *A. avellanae* might be examples of this species.

2. *Larva*. Signoret's proposed differential characters are superficial and of no specific value. On the contrary, the pupae examined from the respective food-plants have now shown identical characters.

Therefore, the identity of both so-called species is established on the characters of the pupae as well as of the adults in general and those of the vasiform orifice and the genitalia in particular. The pupae from hazel, however, often show varied forms because of the presence of the rough surface of the leaves. The descriptions given at the end apply to both forms.

Biology and Food-Plants.

The only adult from the field was collected on oak at Batford, on 1.vi.1937. Some more adults of white-flies were collected from oaks and hazels, both at Bricketwood and Batford, but on examination they were found to be *A. carpini*. During August 1937, a few nymphs in 3rd instar were collected from oaks. At the same time, on 5.viii., one nymph of this species in the 3rd instar was found on hornbeam at Batford. Again on 20.ix. one more nymph in the 3rd instar was collected from hornbeam at Bricketwood. Later, on 11.xi. three pupae of this species were collected from hornbeam from the same locality. It appears evident from these observations that this species breeds in small numbers on hornbeam as well. From the pupae collected on oak and hazel during October and November, 1937, the adults emerged in March and April when kept in a hot-house. This species, therefore, appears to be single-brooded, and overwintering takes place in the pupal stage.

Description of the stages of Pealius quercus ⁴ (Signoret) (fig. 5).

Since all stages were not available, only the three last stages are described with a view to pointing out some of the important features.

Nymph 3rd instar. Greenish-yellow, broadly oval, flat, almost transparent, margin dentate, abdominal segmentation and thoracic tracheal folds faintly visible, measurements 0.64×0.46 mm. Marginal spines three pairs—cephalic and caudo-lateral, sub-equal and anal developed; submarginal spines minute, about 4 or 5 pairs abdominal and 2 or 3 pairs thoracic; dorsal spines three pairs—antennal, thoracic and vasiformal; also 4 or 5 extremely minute and practically imperceptible spines on either side of the median line; ventral spines minute. Eyes entire; legs degenerate; antennae atrophied, directed inwards, hooked at the tip, about 0.02 mm. long. Vasiform orifice relatively small, quadrate, caudal margin almost straight, forming the anterior boundary of a semicircular space posterior to it, measurements about 0.03×0.04 mm.; operculum almost transversely oval, caudal margin hairy, measurements about 0.02×0.03 mm.; lingula small and thick, knobbed distally, hairy all over and with two relatively long hairs at the tip, length about 0.02 mm. and about half exposed beyond the operculum.

⁴ For note on genus see p. 600.

Pupa. Broadly oval, yellow when newly moulted, whitish opaque later on, slightly raised from the leaf surface, enclosed within a wall of wax and a thin layer of wax at the dorsum. Margin raised in a very low ridge all round; submarginal area broad and with punctations which run outward in regular rows; abdominal segmentations distinct, a series of 4 or 5 concolorous warts on either side of the median line; abdominal region separated from the thoracic by a yellowish semicircular ridge; thoracic tracheal folds visible but not prominent on the margin; average measurements 0.88×0.67 mm. Marginal spines, two pairs, anal and caudo-lateral minute; dorsal spines one or two pairs, almost imperceptible; ventrals absent or imperceptible. Legs and antennae as usual, the latter with a small terminal process, about 0.06 mm. long. Vasiform orifice in a depression, the surrounding area forming a pyriform outline, pit ribbed transversely towards the caudal half; orifice proper sub-rectangular, inner lateral margins slightly thickened, average measurements about 0.04×0.04 mm.; operculum slightly brownish, 0.02×0.03 mm., caudal margin hairy; lingula small, knobbed and spinous, the knobbed end exposed beyond the operculum, average length 0.02 mm.

Adult. (Male.) Entirely yellow, genitalia concolorous with the body, tip of rostrum brown; wings pure white, spotless, thin and almost transparent, venation—only the radial sector and a faint cubitus, marginal vein slightly yellowish. Eyes dark brown, constricted in the middle; legs lighter in colour. Antennae relatively small, segments I–III as usual, the 3rd the longest and provided distally with sensoria, IV the smallest, V cylindrical, longest of the distal four segments, provided with a sensorium, VI and VII almost equal, the last segment terminating in a spine. Abdomen tapering posteriorly, 1st abdominal segment transverse, narrow antero-posteriorly, wax plates not marked by coloured margins. Vasiform orifice broader posteriorly, operculum almost sub-rectangular, about 0.02×0.03 mm.; caudal margin arched and hairy with a pair of relatively long hairs on either side; lingula small, relatively broader in the middle. Parameres small, slightly brownish at the tip, and deeply forked, about 0.10 mm. long; aedeagus relatively long, measuring about 0.09 mm.

Female. Concolorous with the male, which it resembles in general characters. Genitalia as usual, dorsal valves with a pair of long prominent hairs near the basal region of the inner valves.

Systematic Position.

The species under discussion are regarded as morphologically identical, and, in the absence of the thoracic tracheal folds ending in a comb of teeth in the pupa, as well as owing to the vasiform orifice being situated in a depression which is transversely ribbed, this species does not fall under the genus *Asterochiton* as proposed by Harrison. On the contrary, these characters ensure a better position for it in the genus *Pealius*, to which it is now assigned. I therefore propose that the species be regarded as *Pealius quercus* (Sign.).

Siphoninus phillyreae (Haliday 1835) and *S. immaculata* (Heeger 1855) (figs. 4, 6).

Haliday (1835) described the species *Aleyrodes phillyreae* and noted some superficial characters of the adult insect. Walker (1852) contributed an almost similar account of the species and briefly mentioned its pupal stage. Frauenfeld (1867) referred to the species but added no information about its immature stages. Signoret (1868) presented a vivid account of the various stages of *A. phillyreae* and regarded the species as distinct from *A. immaculata* which was originally described by Heeger (1855) from *Hedera helix*. He attached considerable significance to the relative number and size of the wax tubes on the pupae and regarded them as of specific value. Douglas (1884), when discussing the species *immaculata*, considered his specimens identical with those of

Heeger, but, in the absence of any specified characters, he was doubtful as to their identity and considered that his specimens might as well be the same as *A. phillyreae* of Signoret. Quaintance and Baker (1914) included both species under the genus *Asterochiton*. Silvestri (1914), however, erected a new genus *Siphoninus* for the species *finitimus* and assigned *A. phillyreae* to it. Some generic characters of the adult, such as the absence of paronychium and the structure of the vasiform orifice both in the adult and the pupa, were discussed. The position of the corresponding species *immaculata* was still undefined. Since then both species have been occasionally referred to by various workers.

Present Investigations.

To test the reliability of the differentiating characters in *S. phillyreae* and *S. immaculata*⁵ proposed by Signoret, observations were made on the relative number and measurements of wax tubes on the pupae of both the species. These results are summarised in Table VIII. The total number of tubes varies from 62 to 74 in *S. phillyreae* and from 61 to 71 in *S. immaculata*. Their lengths also vary from 0.07 to 0.12 mm. and from 0.07 to 0.11 mm., with an average of about 0.10 mm. and 0.10 mm. respectively. As there is considerable overlapping in both the ranges, it is evident that neither of the features can be used with certainty to separate the two forms.

On the other hand, the following differences have been noticed during the present investigations :—

Pupa.

1. *Dorsum*. The shape and colour of the pupae are almost identical, but the pupa of *S. phillyreae* possesses tufts of white waxy material on the dorsum which are not met with in *S. immaculata*.

2. *Wax tubes*. The number and size of these tubes shows no appreciable difference in the two species. The unpaired tube on the 2nd abdominal segment is present in both forms, although Heeger did not show this in his diagram of *A. immaculata*. Nevertheless, their distribution is slightly different—the submarginal series in *S. immaculata* shows, in most cases, more tubes than in *S. phillyreae*; in the median series, on the other hand, the number is invariably higher in *S. phillyreae* (Table VIII).

3. *Vasiform orifice*. The most significant and reliable differences have been noticed in the structure of the vasiform orifice. In *S. phillyreae* (fig. 4) both the operculum and the lingula are smaller in size and the latter does not extend beyond the middle of the orifice. In *S. immaculata*, on the other hand, these structures are relatively bigger and the lingula, which has a conspicuous knob at the caudal extremity, extends considerably beyond the middle of the orifice (fig. 4).

Adult.

In *S. immaculata*, the entire body is uniformly yellow without any grey markings. In *S. phillyreae*, however, the grey bands on the abdomen and the mixture of grey and yellow on the entire body distinguish it readily from the other species.

Nevertheless, in case of any doubt, the vasiform orifice and the genitalia afford contrasting features.

⁵ *A. immaculata* Heeger is assigned to the genus *Siphoninus* Silv. by me.

TABLE VIII.

Number and measurements of the wax tubes on the pupae of *S. phillyreae* and *S. immaculata*.

<i>S. phillyreae</i>								<i>S. immaculata</i>							
No. of the wax tubes on the pupa							Length of the tubes in mm.	No. of the wax tubes on the pupa							Length of the tubes in mm.
Sub-marginal		Median		Inner		Total		Sub-marginal		Median		Inner		Total	
Right	Left	Anterior	Posterior	Thoracic	Abdominal			Right	Left	Anterior	Posterior	Thoracic	Abdominal		
15	14	8-7	6-7	2-2	5-1-4	71	0-10 0-09 0-10 0-10 0-11	19	17	6-6	5-5	2-2	4-1-4	71	0-10 0-11 0-10 0-11 0-10
11	11	6-7	7-7	2-2	4-1-4	62	0-10 0-11 0-11 0-10	18	17	5-5	5-5	2-2	3-1-4	67	0-11 0-10 0-10 0-10
15	14	6-6	7-8	2-2	4-1-4	69	0-11 0-10 0-12 0-08	17	17	5-5	5-4	2-2	4-1-2	64	0-10 0-10 0-10 0-10
13	14	8-7	6-7	2-3	4-1-4	69	0-12 0-08 0-07 0-08	20	18	5-6	5-4	2-2	2-1-3	68	0-10 0-10 0-11 0-11
17	13	6-6	7-7	3-3	4-1-4	71	0-07 0-08 0-09 0-08	15	16	5-6	4-5	2-2	3-1-2	61	0-11 0-11 0-10 0-10
14	15	7-7	7-7	2-4*	4-3-5	74	0-08 0-09 0-09 0-11	16	17	5-5	5-5	2-2	4-1-3	65	0-11 0-11 0-10 0-10
14	12	6-6	6-5	2-2	4-1-4	62	0-08 0-09 0-09 0-11	17	14	5-5	4-3	2-2	4-1-3	60	0-11 0-10 0-10 0-10
13	12	8-7	7-7	3-3	4-1-4	69	0-09 0-11 0-11 0-11	16	18	6-5	4-4	2-2	3-1-4	65	0-11 0-10 0-10 0-10
14	15	8-10	6-6	2-2	4-3-3	73	0-11 0-12 0-11 0-11	15	14	5-5	5-4	2-2	4-1-4	61	0-09 0-09 0-09 0-07
13	14	8-8	7-7	2-3	5-1-4	72	0-11 0-11 0-11 0-11	18	16	5-6	5-5	2-2	4-1-3	67	0-07 0-07 0-07 0-07
14	14	8-7	6-7	2-3	4-1-4	70	0-11 0-10 0-10 0-10	16	17	5-5	5-5	2-2	4-1-4	66	0-07 0-08 0-10 0-10
13	13	7-6	6-6	2-2	4-1-4	64	0-11 0-10 0-11 0-11	18	18	5-5	5-4	2-2	4-1-4	68	0-08 0-10 0-11 0-11
15	13	8-7	6-5	2-2	4-1-5	68	0-10 0-11 0-11 0-11	17	16	5-6	5-4	2-2	4-1-3	65	0-11 0-11 0-11 0-11
14	15	6-7	6-6	2-2	4-1-4	67	0-11	18	18	5-6	5-5	2-2	4-1-4	70	0-11
Average							0-10	Average							0-10

* In this special case there were 1 cephalic, 1 mesothoracic and 2 metathoracic tubes.

i. Vasiform orifice.

In *S. phillyreae*, the lingula is broader at the base, and tapers distally, ending in a narrow tip (fig. 2) and may even be slightly bent. In *S. immaculata*, on the other hand, it is almost uniformly broad and appears as a two-segmented structure (fig. 6).

ii. Genitalia.

Male. The general shape and size of the parameres corresponds in both species. In *S. phillyreae*, however, there is a prominent tooth given out from the ventral surface of the paramere, just posterior to its inner membranous frill. It is differentiated from the claw by a triangular membranous tissue (fig. 2). In *S. immaculata* this tooth is not developed but replaced by an inconspicuous ridge on the ventral surface of the paramere (fig. 6).

Moreover, the aedeagus in *S. phillyreae* ends narrowly and its opening is armed by a pair of minute hooks (fig. 2). These are wanting in *S. immaculata*, but instead, the tip of the aedeagus is provided with a papilla-like outgrowth (fig. 6).

Female. The female genitalia shows but little differentiation except for the presence of a single bristle on either side near the base of the inner valves in *S. immaculata* (fig. 6) against a pair in *S. phillyreae* (fig. 2).

Biology. *S. phillyreae* was collected from *Phillyrea* plants at the John Innes Institution, Merton, Surrey, from which locality Dr. C. B. Williams has also recorded it in 1915. *S. immaculata* was collected from Ivy (*Hedera helix*) at Batford, Herts, in January, 1937.

In both these species, eggs are laid in clusters but not usually in circles, and are profusely dusted with a white waxy powder. The adults of *S. immaculata* are particularly sluggish and are not easily disturbed.

The eggs of *S. phillyreae* laid in the laboratory in the middle of August, 1937, hatched in about 16 days and the overwintering was in the nymphal stages. The emergence of the adults commenced from 16th May 1938. During 1937, however, some material was obtained from the John Innes Institution and Camberley. In both these cases the adults emerged during August and September and laid eggs in the laboratory.

S. immaculata is single-brooded. Nymphs collected in the field in January, 1937, were in most cases of the first instar. They were kept in a hot-house and adults emerged in April. Eggs were laid on ivy⁶ in captivity and when kept in the same hot-house, they hatched after 35–39 days. The plants with the nymphs on them were removed to the insectary in June, 1937, but the life-cycle was not completed till May of the next year.

Description of the various stages of Siphoninus phillyreae (Haliday) (figs. 2, 4).

Since neither of these two species had been properly described, it was considered advisable to describe *S. phillyreae* and note the specific differences for the other species.

Egg. Oblong, pedunculate, yellow when freshly laid, changes to brown subsequently; measurements about 0.26×0.09 mm., stalk about 0.03 mm.

Nymph 1st instar. Elliptical, may be slightly elongated, a little convex dorsally; brownish,⁷ more so in the middle; dusted profusely with the white waxy powder; fringe of white waxy plates all round; average measurements 0.30×0.17 mm. Margin with minute projections which indicate the bases of the marginal spines, of which there are sixteen pairs; submarginal area transversely striated. Dorsal spines minute, three pairs—antennal, abdominal and vasiformal; ventrals—rostral and vasiformal. Eyes generally entire; legs functional, three segmented. Antennae long and slender; apparently four segmented, average measurements 0.07 mm. Vasiform orifice close to the caudal margin, subcordate, average measurements 0.02×0.02 mm.; operculum sub-rectangular, measuring approximately 0.01×0.01 mm.; lingula short, setose, about 0.01 mm., mostly exposed.

Nymph 2nd instar. Oval; colour, shape and the fringe as in the previous instar, average measurements 0.41×0.25 mm. Margins slightly dentate; marginal spines four pairs—one cephalic and three caudals, the anals being the longest; a submarginal series of about eleven or twelve pairs of extremely minute spines; dorsal spines as in the previous instar; a single pair of ventral spines vasiformal in position. Six pairs of dorsal wax tubes—1 cephalic, 1 optic, 2 thoracic and 2 abdominal, running parallel to the margin. Eyes relatively small, legs degenerate, antennae atrophied about 0.02 mm. long, directed backward. Vasiform orifice sub-circular, posterior margin transversely notched, inner lateral margins slightly thickened, average measurements 0.03×0.03 mm.; operculum sub-rectangular, about 0.01×0.02 mm., caudal margin hairy; lingula cylindrical, spatulate towards the tip, about 0.01 mm. long and more than half exposed beyond the operculum.

Nymphal 3rd instar. The shape, colour and the marginal and submarginal spines as

⁶ Two attempts were made to transfer adults of *S. immaculata* to *Phillyrea*, but in both cases the females died without laying eggs.

⁷ Soon after hatching and moultings the colour of the nymph is yellow.

in the previous instar, waxy fringe narrower, average measurements 0.59×0.41 mm., dorsal spines comparatively better developed. Legs⁸ degenerate, antennae⁸ directed inward, hooked at the tip, length about 0.03 mm.; eyes small, entire. Dorsal wax tubes roughly in three series: (1) submarginal, 9 or 10 pairs—2 or 3 cephalic, 3 thoracic and 4 abdominal; (2) median, about 4 pairs—2 or 3 abdominal and 1 or 2 thoracic; (3) inner—2 thoracic and 1 abdominal. Vasiform orifice sub-circular, caudal half showing foam-like chambers; operculum 0.01×0.02 mm.; lingula about 0.02 mm., about $\frac{2}{3}$ exposed.

Pupa. Elliptical, raised within the closely surrounding ring of wax. Dorsum with tufts of white waxy material. Colour hazel brown outside, dark brown in the middle. Average measurements 1.02×0.74 mm. Margin lightly crenulate, caudal fold prominent with a row of teeth at the margin. Marginal spines absent; submarginals 14 or 15 pairs; dorsal spines three pairs, ventral spines wanting or a minute pair vasiformal in position. Dorsal tubes practically in three series but considerably increased in numbers—submarginal 11–15 pairs, often showing asymmetry in number on either side; median a little irregular in arrangements, 12–15 pairs; inner generally with 2 or 3 pairs of thoracic, 4 abdominals often showing asymmetry in their numbers and a single additional tube in the 2nd abdominal segment and occasionally 1 or 2 more on the posterior segments. The total number of these tubes ranges between 62 and 74. Legs and antennae as usual in the instar, the latter with a fine terminal process and measuring, on an average, 0.07 mm. Vasiform orifice sub-circular, slightly thickened at the margins, caudal half showing foam-like chambers, average measurements 0.06×0.05 mm.; operculum transversely rectangular, average measurements 0.01×0.03 mm.; lingula short and narrow, knobbed at the tip, about 0.02 mm. long and $\frac{2}{3}$ exposed beyond the operculum.

Adult. (Male) yellow, head and thorax uniformly yellow, metathorax often with a faint greyish mark. Eyes constricted, antennae seven segmented, greyish distally from the middle of the 3rd segment, segment III the longest, often showing variations in measurements, IV the smallest, V longer than IV, VI or VII. Tip of rostrum dark brown. Legs greyish distally, paronychium absent. Wings white, spotless, smoky towards the blade, outer marginal vein shining yellow, median vein darker distally. Abdomen yellow, dark bands on the 2nd abdominal segment prominent; margins of the wax plates dark; dorsally a few transverse bands of brown. The genitalia and the last abdominal segment grey. Vasiform orifice almost circular; operculum sub-rectangular; lingula long, narrowing distally (fig. 2), may be slightly bent, average length 0.03 mm., parameres well developed, each with a prominent tooth towards the distal end (fig. 2), average length 0.15 mm.; aedeagus with a pair of chitinous hooks at the tip, length 0.11 mm. (fig. 2).

(Female) concolorous with the male, but abdomen generally lighter in colour and with fewer cross bands; genitalia acute (fig. 2).

Dialeurodes chittendeni Laing 1928 (fig. 7).

The species was first recorded from *Rhododendron* by Hoare (1928), who doubtfully regarded it as belonging to *Pealius*. Laing (1928) named the species and described the characters of the pupa-case and adults. The generic position was assigned provisionally to *Dialeurodes* which is still maintained. Fox-Wilson (1929 and 1935) gave a brief account of this species as a pest of rhododendrons and mentioned briefly the immature and the adult stages with some remarks on the life-history. Latta (1937) published a brief note on this species as a pest of rhododendrons in the United States.

⁸ Silvestri (1914) did not describe the nymphs of *S. finitimus* regarded as the genotype. Lately, however, Priesner and Hosney (1932) described the immature stages of an allied form *S. granati*. They have stated that the antennae and legs are wanting in the 2nd and 3rd instars. This does not agree with the present observations. These organs have been noticed in all species studied so far and are also present in *S. phillyreae*, as stated above.

The various stages of this pest are described in detail because a fuller account was needed.

Egg. Elongated, concave on one side, surface reticulate, peduncle long; inserted a little, shifted from the basal end; average measurements 0.24×0.11 mm., stalk 0.06 mm.; whitish when fresh, turns to brownish subsequently. The eggs are laid singly without any waxy powder dusted at the spot.

Nymph 1st instar. Greenish-yellow, concolorous with the leaf surface, elliptical, devoid of the waxy fringe, measures about 0.34×0.20 mm. Marginal spines 18 pairs—3 cephalic, 7 thoracic, 6 abdominal and 2 caudal; fifteen of these on either side arise from minute papillae the bases of which appear just submarginal in position; two pairs of

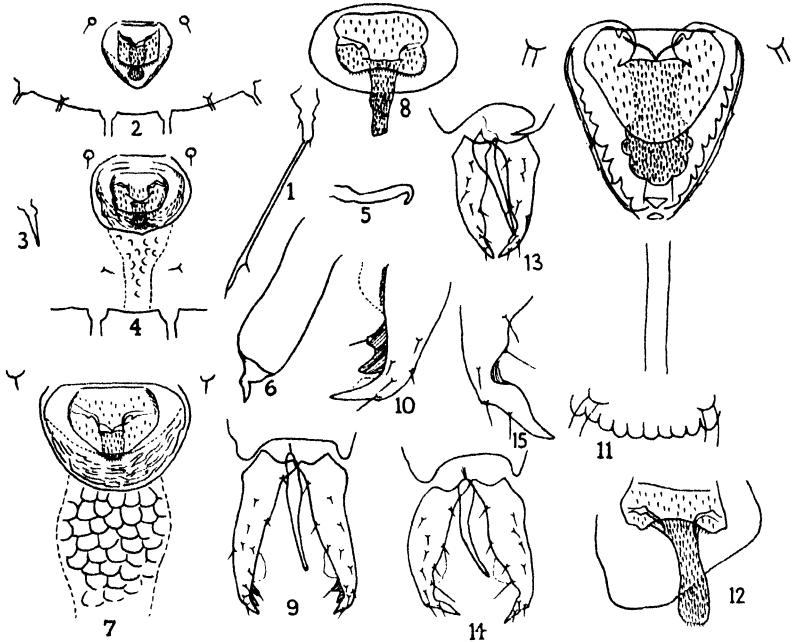


FIG. 7.—1-10, *D. chittendeni*, 1, antenna of 1st instar larva; 2, vasiform orifice of 1st instar larva; 3, antenna of 2nd instar larva; 4, vasiform orifice of 2nd instar larva; 5, antenna of 3rd instar larva; 6, antenna of pupa; 7, vasiform orifice of pupa; 8, vasiform orifice of adult; 9, genitalia of male; 10, tip of a paramere; 11-13, *T. vaporariorum*, 11, vasiform orifice of pupa; 12, vasiform orifice of adult; 13, genitalia of male; 14-15, *T. sonchi*, 14, genitalia of male; 15, tip of a paramere.

minute dorsal spines—antennal and vasiformal; two pairs of minute ventral spines—rostral and vasiformal. Eyes entire; antennae about 0.09 mm., distal segment ending in a spine; legs functional. Vasiform orifice cordate, measurements 0.02×0.03 mm.; inner lateral margins slightly thickened, operculum almost sub-squarish, about 0.01×0.02 mm.; caudal margin hairy, lingula small, 0.01 mm., knobbed distally.

Nymph 2nd instar. Greenish-yellow, almost transparent, elliptical but more rounded, without a waxy fringe, entire surface minutely papillate, measures about 0.45×0.31 mm. Marginal spines three pairs—cephalic, caudo-lateral, and anal; 12 or 13 pairs of extremely minute submarginal spines; three pairs of minute dorsal spines and two of ventral. Eyes entire; legs degenerate; antennae atrophied, directed backwards, about 0.02 mm. long. Vasiform orifice a little transversely elliptical, inner lateral margins slightly thickened,

measurements about 0.03×0.04 mm.; operculum almost similar, 0.02×0.02 mm.; lingula small, cylindrical, a little knobbed and exposed at the end, length about 0.01 mm.

Nymph 3rd instar. Shape and colour as in the previous instar; devoid of any waxy fringe, dorsum minutely papillate, average measurements 0.77×0.58 mm. Marginal spines 17 pairs, the lateral cephalic and the anal prominent; dorsal and ventral spines as in the previous instar. Eyes entire; legs degenerate; antennae atrophied, directed inward and hooked at the tip, 0.03 mm. long. Vasiform orifice as in the previous instar.

Pupa. Broadly elliptical, 1.15×0.90 mm., greenish pale-yellow, submarginal area whitish, margin entire; devoid of waxy fringe, dorsum minutely papillate. Marginal spines 10–15 pairs—cephalic, caudo-lateral and anal being truly marginal and prominent, others minute, and slightly submarginal in position. Dorsal and ventral spines as in the previous instars. Caudal furrow prominent, showing cellular markings. Eyes well developed, legs and antennae as usual in this instar, the latter terminating in a small process and measuring about 0.08 mm. Vasiform orifice relatively small, almost semi-circular, enclosed in a corresponding marginal area running close together, broader than long, inner lateral and caudal margins thickened, posterior half of the floor thickened by irregular, transverse ridges, average measurements 0.04×0.06 mm.; operculum similar in shape, cephalic margin straight, average measurements 0.02×0.03 mm.; lingula very small, almost entirely obscured by the operculum; length about 0.01 mm.

Adults. Male. Light-yellow, body without any sculpture, tip of rostrum brown; eyes constricted, facets sub-equal; wings white, spotless. Antennae concolorous with the body, seven segmented, about 0.32 mm. long, segment III the longest, IV the smallest from II to VII, VII longer than any from IV to VI; measurements of the antennal segments variable. Vasiform orifice transversely oval; operculum sub-quadrate, about 0.02 mm., caudal margin hairy; lingula long and broad, and about 0.02 mm. long. Parameres long and stout, length about 0.16 mm.; two teeth-like prominences sub-apically, the distal one narrower apically and the proximal one slightly broader at the apex and provided with a small hair; aedeagus shorter than the parameres.

Female. Concolorous with the male, to which it agrees in general characters. Antennae about 0.40 mm., segmental measurements variable, and occasionally segment V may be longer than VII. Genitalia as usual.

Tetralicia ericae Harrison 1917 (fig. 1).

Harrison (1917) described briefly the adult, larva and the pupa of this species and included it in *Tetralicia*. Baker and Moles (1921) and Singh (1931) included this genus in their classification.

I have not been able to collect an adult, although large quantities of heather (*Erica tetralix*) were examined from Hindhead, Surrey, and Wheathampstead, Herts. A few specimens of nymphs and pupae, however, were collected and their descriptions added since Harrison and subsequent authors did not describe these stages in detail.

Nymph 2nd instar. Elongated but slightly narrower at the extremities. Colour dark,⁹ margins slightly deflexed ventrally; measurements about 0.46×0.20 mm. Abdominal segmentation distinct; mounted specimens show a series of star-shaped corrugated structures on either side of the median line (these might be some protuberances on the dorsal surface). Marginal spines three pairs—cephalic, caudo-lateral and anal, and a series of 5 or 6 minute submarginal spines. There are some minute circular pores towards the ends of the intersegmental partitions. Two pairs of dorsal spines—antennal and vasiform. Legs degenerate; antennae atrophied, directed backwards. Vasiform

⁹ Harrison described the colour of the larva as transparent but whitish. This was probably the case of a newly moulted nymph.

orifice almost circular, about 0.04×0.04 mm.; with a corresponding fold-like boundary outside, inner margins thickened all round; operculum subcordate, filling almost the entire orifice, measuring about 0.03×0.03 mm.; lingula small, a little knobbed distally, obscured by the operculum, length about 0.02 mm.

Nymph 3rd instar. Corresponds with the previous instar, in shape, structure and colour, measurements about 0.68×0.29 mm. The submarginal spines not prominent, segmental star-shaped structures quite distinct. Legs degenerate, antennae minute, narrower distally and directed inward, about 0.01 mm. Structure of the vasiform orifice as in the previous instar, measurements about 0.04×0.04 mm.; operculum, slightly notched inwards on the lateral margins, measures about 0.03×0.03 mm.; lingula, entirely obscured by the operculum, length about 0.02 mm.

Pupa. Elongated, narrower at both ends, about 1.03×0.56 mm.; margin deflexed, submarginal area smooth. Colour black.¹⁰ A median row of circular areas in abdominal region. Legs and antennae as usual in the instar, the latter without terminal process, length about 0.07 mm. Vasiform orifice almost circular, enclosed within a concentric area, inner margins very much thickened, the lateral ones with a series of broad and blunt teeth, measurements about 0.06×0.05 mm.; operculum 0.04×0.04 mm.; lingula entirely obscured by the operculum, length 0.03 mm.

Trialeurodes vaporariorum (Westwood 1856) (fig. 7) and *T. sonchi* Kotinsky 1907 (fig. 7).

T. vaporariorum is a widely spread species and therefore it has already received considerable attention, particularly in relation to glass-house horticulture. Westwood (1856) described the species and mentioned its food-plants. Hargreaves (1915) published an account of its structure and biology. Lloyd (1922) supplemented this by giving its habits and methods of control, while Weber (1935) contributed to its morphology.

T. sonchi was originally described by Kotinsky (1907). Lloyd (1922) recorded it from greenhouses in England in association with the former species. Deshpande (1933) added a brief description of all stages.

Present Observations.

Two types of pupae were met with in greenhouses—(1) with 5–8 pairs of dorsal wax tubes and relatively shorter marginal wax processes, (2) without the dorsal wax tubes but with relatively longer marginal wax processes. This second type was identified by Mr. F. Laing as *T. sonchi* (vide Lloyd 1922). The pupae with the dorsal wax tubes (typical *T. vaporariorum*) have been found mostly associated with the hairy leaves particularly of *Nicotiana tabacum*, and *Nicotiana glutinosa*, while the other type was specially very common on the smooth leaves of *Nicotiana glauca*, even when these plants were in the same greenhouse.

The pupae of both types were also met with on hollyhock (*Althea rosea*) in nature as previously recorded by Lloyd. In one case on 5.xi.1936, *T. vaporariorum* was found breeding on a wild plant *Solanum dulcamara*, in the fields at Batford, Herts, far removed from greenhouses.

The pupae collected from *Sonchus* sp. in the field were without the dorsal wax tubes as stated by Deshpande (1933) but in general structure they corresponded with those of the other type.

The adults are practically identical, except that sometimes those of *T. vaporariorum* are uniformly yellow in colour. It is, however, not possible

¹⁰ The descriptions of the stages given above are based on mounted specimens after bleaching.

to express an opinion on the adults from both types of pupae. The adults from *Sonchus* sp. and those bred from the pupae had invariably grey antennae, legs and the abdominal end. In two cases only the males from *Sonchus* sp. showed slightly different genitalia (fig. 7), in which case the parameres were longer than in the other form (fig. 7) and the aedeagus was also relatively smaller in relation to the parameres. It seems probable that adults with genitalia differing from the typical forms represent the species *T. sonchi*.

Aleyrodes fragariae Walker 1852.

The distinctiveness of this species seems a little doubtful. The specimens of the adults collected from strawberries locally, as well as those received from Dr. Massee from East Malling, yielded mixed individuals of *A. proletella* and *A. loniceræ*.

It was possible to breed both these species on strawberries in the laboratory as already described, but no immature stages were collected from this plant in nature.

The conclusive evidence to doubt the significance of this species was obtained from the breeding experiments with the adults received from Dr. Massee on 23.ix.1937. The progeny of the overwintering females was a mixed population of the yellow and grey individuals in the beginning of May 1938, and then of entirely yellow ones towards its latter half.

Since these adults behaved exactly as those of *A. loniceræ* in the laboratory, it is possible that the same species may have a wider range of food-plants. However, in the absence of any immature stages in the field on strawberries it is not justifiable to express an opinion in this respect.

List of British species of ALEYRODIDAE.

According to the present investigations, the following is the list of the species of ALEYRODIDAE known from, or suspected to exist in, Great Britain :—

(A) *Examined during the present investigations.*

- (1) *Aleyrodes proletella* Linn. = (*brassicæ* Walker).
- (2) *Aleyrodes loniceræ* Walker = (*rubi* Signoret).
- (3) *Aleyrodes fragariae* Walker—Doubtful.
- (4) *Asterobemisias carpini* Koch = (*rubicola* Douglas).
- (5) *Pealius quercus* Signoret = (*avellanae* Signoret).
- (6) *Siphoninus phillyreæ* Haliday.
- (7) *Siphoninus immaculata* Heeger.
- (8) *Dialeurodes chittendeni* Laing (Note 1).
- (9) *Tetrallia ericæ* Harrison.
- (10) *Trialeurodes vaporariorum* Westwood (Note 2).
- (11) *Trialeurodes sonchi* Kotinsky (Note 2).
- (12) *Trialeurodes williamsi* Trehan (Note 2).
- (13) *Aleuroplatus kewensis* Trehan (Note 2).

(B) *Not seen during the present investigations.*

- (14) *Aleyrodes ribium* Douglas.
- (15) *Aleyrodes spiracæ* Douglas.
- (16) *Aleyrodes filicium* Goeldi (Note 3).
- (17) *Aleyrodes azaleæ* Baker and Moles (Note 4).

Notes.

- (1) Introduced species now found out-of-doors on rhododendrons.
- (2) Introduced species found in greenhouses; *T. vaporariorum* occasionally survives the summer out-of-doors, but not, so far as I know, the winter.
- (3) Reported from ferns at Kew by Douglas but probably a misidentification.
- (4) Taken on imported azaleas in a greenhouse near Edinburgh by V. G. Deshpande in November 1930 (unpublished thesis in the Library of the University of Edinburgh).

*Key for identification of the species studied.**Pupae.*I. *Elevated from the leaf surface.*

A. Highly elevated, box-shaped, outer wall of wax conspicuous.

1. Hazel colour; vasiform orifice almost rounded, inner margins thickened, caudal portion showing foam-like chambers; operculum sub-rectangular, cephalic margin removed from that of the orifice.

Dorsal wax tubes present in large number.

- i. Dorsum with tufts of white waxy material, operculum and lingula small, the latter knobbed at the end and without prominent terminal hairs, lingula extending to about the middle of orifice *S. phylliracae*.
- ii. Dorsum without tufts of white waxy material, operculum and lingula relatively large, lingula knobbed distally and with a pair of long prominent hairs at the tip and extending to about $\frac{2}{3}$ of the orifice *S. immaculata*.

2. Whitish in colour, series of marginal wax processes, vasiform orifice subcordate, inner lateral margins toothed, operculum semicircular, cephalic margin almost conjointly parallel to that of the orifice, lingula lobed distally and armed with a pair of prominent hairs at the tip.

- i. Dorsum with about 5-8 pairs of dorsal wax tubes, marginal wax processes relatively small *T. vaporariorum*.
- ii. Dorsum without any dorsal wax tubes, marginal wax processes relatively long *T. sonchi*.

B. Slightly elevated, enclosed in a closely fitting wall of wax.

- i. Greenish-yellow, 26-32 crescent-shaped submarginal papillae, giving rise to tape-like waxy tubes, a pair at each extremity relatively longer and prominent. Tracheal folds ending in a comb of 3 or 4 teeth; vasiform orifice subcordate, cephalic margin rounded and posteriorly a prominent caudal furrow, lingula lobed, $\frac{2}{3}$ of the lobed end exposed *T. williamsi*.
- ii. Yellowish-opaque, margin with a slightly raised ridge all round, abdominal segments with concolorous warts on either side of the median line, submarginal area broad and punctated; vasiform orifice in a depression which is ribbed transversely, orifice relatively small, sub-rectangular; lingula small and knobbed *P. quercus*.

II. *Not elevated from the leaf surface.*

A. Tracheal folds not evident on the margin.

1. Dorsum convex.

a. Sides fixed to the leaf by a border of wax.

- i. Yellow or dark brown; vasiform orifice subcordate, inner lateral and caudal margins uniformly thickened, lingula spatulate with a pair of long hairs at the tip, about half exposed . . . *A. proleptella*.
- ii. White, yellow or golden, abdominal region with concolorous circular areas on the median line; vasiform orifice subcordate, inner lateral margins toothed, lingula spatulate, relatively broader towards the caudal half and with a pair of long terminal hairs
A. lonicerae.

b. Sides fixed to the leaf without a border of wax.

- i. Yellowish-white, dorsum with minute papillae; caudal furrow with cellular markings; vasiform orifice semicircular, inner lateral and caudal margins thickened, posterior half of the floor with irregularly transverse ridges; operculum similar, lingula small, just the tip visible *D. chittendeni*.

2. Dorsum deflexed.

- i. Black, vasiform orifice circular, surrounded by a similar area, inner lateral margins thickened with a series of broad teeth, lingula entirely obscured by the operculum *T. ericae*.

B. Tracheal folds evident and ending in a comb of teeth.

- i. Whitish-opaque, outline of developing insect marked by an oval formed by concolorous warts; vasiform orifice long and triangular, inner lateral margins toothed, operculum semicircular, lingula long, thick and spatulate, with a pair of long hairs at the tip and more than half exposed *Asterobemisia carpini*.
- ii. Yellow, dorsum with a low keel in the median cephalic and thoracic region; vasiform orifice elevated and bounded by brownish ridges, sub-circular, inner row of striations prominent, operculum large, lingula stout, inverted half-opened mushroom-shaped, head spinous and with a pair of stoutish hairs at the tip, the head almost exposed *Aleuroplatus kewensis*.

*Adults.*I. *Wings spotted.*

1. Fore-wings with two grey spots.

Adults spotted with brown on head and thorax, transverse bands on the dorsum of the abdomen; operculum sub-rectangular, lingula long and thick, hairy at the tip; aedeagus angularly bent, parameres with an outgrowth sub-apically *A. proleptella*.

2. Fore-wings with one grey spot.

Adults pure yellow or spotted with brown at the head, thorax and legs, etc., also the genitalia grey, operculum notched at the caudal margin, lingula long, aedeagus slightly curved; parameres with a heel-shaped structure sub-apically *A. lonicerae*.

II. *Wings spotless.*

1. Fore-wings slightly smoky towards the blade: body with a mixture of grey and yellow; operculum rectangular, lingula long and tapering distally, may be slightly bent; aedeagus with a pair of hooks at the tip; parameres long and stout, each with a sub-apical tooth *S. phillyreae*.
2. Wings clear.
 - i. Adults pure yellow; operculum rectangular, lingula long and broad and appears as if two-segmented, parameres long and stout, each with a sub-apical ridge on the ventral side; aedeagus with a papilla-like outgrowth at the tip *S. immaculata*.
 - ii. Adults yellow; operculum quadrate, lingula long and uniformly broad, aedeagus almost straight, parameres long and stout, each with two sub-apical inner teeth-like prominences, the distal one narrower apically and the proximal one slightly broader and with a hair at the apex *D. chittendeni*.
 - iii. Adults yellow, operculum sub-rectangular, lingula small, narrower towards the tip; aedeagus relatively long, reaching near the tip of the parameres; parameres small, and deeply forked at the tip *P. quercus*.
 - iv. Adults yellow, operculum notched at the caudal margin, lingula small and broad, rounded at the tip, parameres medium *Asterobemisia carpini*.
 - v. Adults yellow: operculum rectangular, lingula small, broader at the tip, parameres small, rounded at the apex and provided with a claw-shaped structure at the tip *T. williamsi*.
 - vi. Adults yellow; operculum transverse, arched at the caudal margin, lingula long, narrow and with a few hairs at the tip, parameres small, with an inner row of seven teeth increasing in size distally *Aleuroplatus kewensis*.
 - vii. Adults yellow or with the distal part of antennae, abdominal end and legs grey, operculum transverse, lingula long, slightly broader at the tip, parameres medium, aedeagus relatively long and reaching near the tip of the parameres *T. vaporariorum*.
 - viii. Adults yellow with distal part of antennae, legs and abdominal end grey, operculum and lingula as above, parameres relatively long with bent tips and aedeagus smaller than the parameres *T. souchi*.

Parasites recorded.

The following is the list¹¹ of the parasites bred from the pupae of the respective hosts collected in the field :—

Parasite	Host
<i>Encarsia partenopea</i> Masi.	<i>S. phillyreae</i> and <i>S. immaculata</i> .
<i>Aleurodiphagus</i> ? <i>clavicornis</i> Thoms.	<i>A. lonicerae</i> , <i>A. rubi</i> ¹² and <i>A. carpini</i> .
<i>Isostasius</i> sp.	<i>A. carpini</i> .
<i>Eretmocerus</i> sp.	<i>A. carpini</i> .
<i>Encarsia formosa</i> Gahn.	<i>T. vaporariorum</i> .
<i>Prospeltella</i> sp.?	<i>T. williamsi</i>
<i>Eretmocerus corni</i> Hald.	<i>P. quercus</i> .

¹¹ The parasites were kindly identified by Dr. Ch. Ferrière of the British Museum (Natural History).

¹² Pupae of *A. lonicerae* type collected from blackberry.

Parasitisation in nature was studied in three species : namely, *S. phillyreae*, *S. immaculata* and *A. carpini*, and the following observations were made :—

(1) *S. phillyreae*.

A. John Innes Institution, Merton (Surrey).

- (i) May 1937. Out of 225 pupae examined, 38 were found parasitised.
- (ii) June 1937. Out of 65 pupae 23 were found parasitised.

Average parasitisation, therefore, was 21%.

B. Camberley (Surrey).

- (i) September 1937. Out of 141 pupae examined, 53 were found parasitised.
- Percentage of parasitisation was 37·6%.

Average percentage of parasitisation in the species from both the localities was 26·5%.

(2) *S. immaculata*.

Batford (Herts).

- (i) February 1937. Out of a total of 250 pupae and pupae cases, 72 were found parasitised. This gave 28·8% parasitisation.

From both these species *Encarsia parthenopea* Masi was bred out in the laboratory.

(3) *Asterobemisia carpini*.

Batford (Herts).

- (i) April 1937. Out of 42 pupae examined, 15 were found parasitised.
- (ii) May 1937. Out of 51 pupae 22 were parasitised.

Average parasitisation, therefore, was 39·8%.

- (iii) March 1938. 16 parasitised out of 39 pupae.
- (iv) April 1938. 19 parasitised out of 47 pupae.
- (v) May 1938. 13 parasitised out of 33 pupae.

Average parasitisation 40·3%.

Average parasitisation from two years' observations, therefore, comes to 40·1%. The species of the parasites bred out from these collections are given in the list.

Oviposition. This was studied in *Encarsia parthenopea*, under a binocular and the following observations made :—

The parasite surveyed the host thoroughly with the help of its antennae, which worked very briskly during the act. When satisfied about the host and the proper position, the parasite balanced its body on its legs, shot out the ovipositor and inserted it in the host. The insertion is generally very deep and the body of the parasite often rests on that of the host when penetration has taken place. During this stage the antennae were stationary and bent almost at right angles to the head. The duration of the act varied each time. In four cases under observation, the duration varied from 1·5 minutes to 9 minutes with an average duration (from 48 observations) of about 4 minutes and 52 seconds.

The parasite repeated these operations very frequently, one following the other, but after a few stings it often rested a while before starting again.

In one case the parasite was noticed working its mouth-parts on the puncture made by the ovipositor, and took about 19 minutes over it. This, however, is not a common practice, as also stated by Imms (1916), and it is quite probable that the parasite utilises the fluid that oozes out of the puncture, when a feed is required.

During these observations a parasite was observed to puncture the same pupa in three different places, but in no case did more than one parasite emerge from a parasitised pupa. Further, it has also been observed that, when a full-developed pupa was punctured by a parasite, the adult of the host white-fly emerged out invariably. Hence it proved that mere puncturing did not result in killing the developing host. This does not agree with Speyer (1927).

Life-cycle and transfers of some of the parasites from one host to the other.

Attempts were made to breed the parasites in the laboratory with a view to finding out the possibility of some interrelation of parasites with hosts other than those specific to them. The results of these observations are given in Table IX. These trials proved successful only with the parasites from *S. phillyreae* (*Encarsia partenopea* Masi) and *T. vaporariorum* (*Encarsia formosa* Gahn.) which bred readily on *A. proletella*. This Aleyrodid, therefore, may be considered a most suitable host for the interbreeding of these parasites. Speyer (1927) stated that the normal life-cycle in *E. formosa* did not take fewer than 28 days. The present observations support this.

TABLE IX.

Life-cycle and transfers of some of the parasites of white-flies from one host to the other.

Date.	From	On	Duration of life-cycle in days	Remarks.
1937				
16.v.	<i>A. carpini</i>	<i>A. carpini</i>	38-46	Successful.
25.v.	<i>S. phillyreae</i>	<i>A. carpini</i>	—	Not successful.
25.v.	<i>S. phillyreae</i>	<i>T. vaporariorum</i>	—	Not successful.
29.v.	<i>S. phillyreae</i>	<i>T. vaporariorum</i>	—	Not successful.
29.v.	<i>S. phillyreae</i>	<i>T. vaporariorum</i>	—	Not successful.
25.vi.	<i>A. carpini</i>	<i>A. proletella</i>	—	Not successful.
25.vi.	<i>A. carpini</i>	<i>A. proletella</i>	—	Not successful.
30.vi.	<i>A. carpini</i>	<i>A. proletella</i>	—	Not successful.
2.vii.	<i>S. phillyreae</i>	<i>T. vaporariorum</i>	—	Not successful.
13.viii.	<i>S. phillyreae</i>	<i>A. proletella</i>	30-36	Successful.
14.viii.	<i>S. phillyreae</i>	<i>T. vaporariorum</i>	—	Not successful.
22.viii.	<i>T. vaporariorum</i>	<i>T. vaporariorum</i>	33-40	Successful.
29.viii.	<i>T. vaporariorum</i>	<i>T. vaporariorum</i>	35-37	Successful.
29.viii.	<i>T. vaporariorum</i>	<i>A. proletella</i>	32-35	Successful.
7.ix.	<i>S. phillyreae</i>	<i>A. proletella</i>	31-33	Successful.
8.ix.	<i>S. phillyreae</i>	<i>A. proletella</i>	29-32	Successful.
7.ix.	<i>S. phillyreae</i>	<i>A. carpini</i> & <i>A. lonicerae</i>	—	Not successful.
28.ix.	<i>A. lonicerae</i>	<i>T. vaporariorum</i>	—	Not successful.
1938				
?	<i>T. vaporariorum</i>	<i>A. lonicerae</i>	?	Successful. ¹

¹ During March and April 1938, a number of parasites of *T. vaporariorum* (*Encarsia formosa* Gahn.) were bred out from the pupae of *A. lonicerae* which were kept in a hot-house where *T. vaporariorum* and its parasites were also present.

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221. *The Active Principles of Leguminous Fish-poison Plants.*
Part V. Derris malaccensis and Tephrosia toxicaria.

By STANLEY H. HARPER.

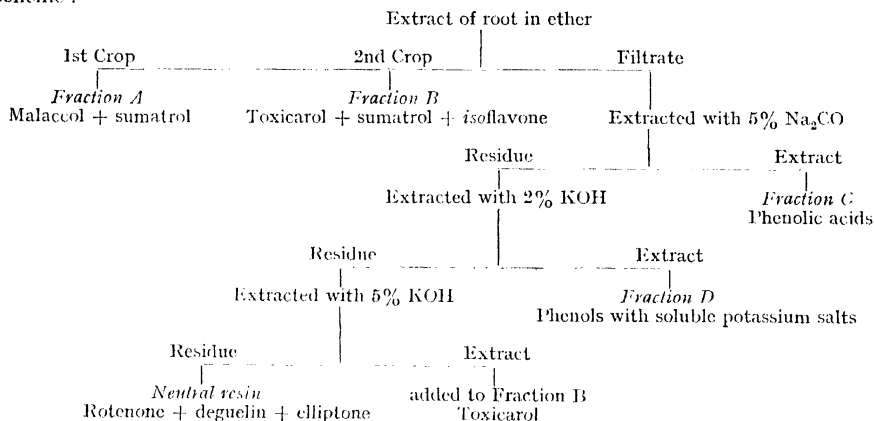
The resin from *D. malaccensis* root has been fractionated by chemical means, and *l*- α -toxicarol obtained in a pure condition. In addition rotenone, elliptone, deguelin, malaccol, sumatrol, and a new phenol have been isolated. The properties of this

phenol, which is isomeric with toxicarol, are discussed and as a working hypothesis an isoflavone structure (IV) is suggested.

The resin from *T. toxicaria* root has been similarly fractionated, and rotenone, *l*- α -toxicarol, and sumatrol isolated.

In Part I (J., 1939, 812) the isolation of *l*- α -toxicarol from *Derris malaccensis* root and its separation from sumatrol was examined, the properties of the substance agreeing with those recorded by other workers (Tattersfield and Martin, *J. Soc. Chem. Ind.*, 1937, 56, 77r; Cahn, Phipers, and Boam, J., 1938, 513). It was pointed out, however, that it did not behave on analysis as a pure substance, particularly in view of its high methoxyl content, and the suggestion was made (*Chem. and Ind.*, 1938, 57, 451) that a third substance might be present. Cahn, Phipers, and Boam (*loc. cit.*) too had suggested the possibility of a third substance, other than sumatrol, in the crude product. Subsequently malaccol was isolated in small yield from this root (Meyer and Koolhaas, *Rec. Trav. chim.*, 1939, 58, 207; Harper, this vol., p. 309), although separating in a different fraction to the toxicarol, and it was uncertain to what extent this affected the previous findings. From the experience gained and the material accumulated in the work on malaccol it has been possible to re-examine successfully the question of the homogeneity of *l*- α -toxicarol.

The ethereal extract of the root was therefore fractionated according to the following scheme:



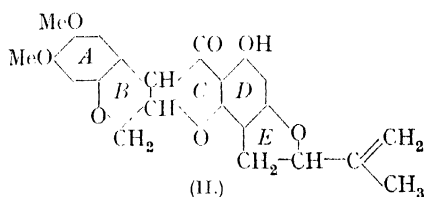
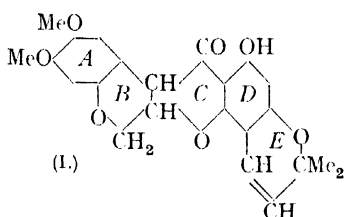
The first precipitate, which was gelatinous and separated before the crystalline toxicarol, was shown (this vol., p. 309) to contain malaccol, and the acetone filtrate from this has deposited only sumatrol on keeping for as long as six months in the refrigerator. The second precipitate of crude toxicarol constituting the main bulk of the resin was examined in detail as described below. When on concentration of the ethereal solution and refrigeration no further toxicarol would separate, the solution was extracted first with sodium carbonate to give a fraction of phenolic acids and secondly with 2% potassium hydroxide solution, which separated a phenolic fraction. This was distinct from toxicarol because it gave a soluble potassium salt and failed to crystallise from ether; these fractions were not, however, examined in detail. Subsequent extraction with 5% potassium hydroxide solution gave the characteristic insoluble yellow salt of toxicarol, which was acidified under ether, and the toxicarol that crystallised added to that obtained previously. The ethereal extract then gave on evaporation the neutral resin, which, by the method elaborated for the separation of elliptone (J., 1939, 1099), was shown to contain rotenone, deguelin, and elliptone. Neither of the last two has previously been reported as occurring in *D. malaccensis*. An account of this is, however, reserved for a subsequent communication.

The crude toxicarol was first fractionated from ethyl acetate solution. After ten crystallisations a series of head fractions of toxicarol of constant specific rotation ($[\alpha]_D^{20}$ - 67° in benzene) were taken off until no more would separate. This material, although

bright yellow and devoid of the greenish tinge reported previously (Cahn, Phipers, and Boam, *loc. cit.*), was expected to contain sumatrol. These head fractions were therefore fractionated from ether by the method described previously (J., 1939, 812). The fractions obtained, however, had the same specific rotation and both sumatrol and malaccol, which is also sparingly soluble in ether, were absent. This material was thus homogeneous and therefore pure *l*- α -toxicarol. The pure *l*- α -toxicarol so obtained, giving the correct analysis for $C_{23}H_{22}O_7$, crystallised from ether and ethyl acetate-alcohol in bright yellow laths in an indefinitely solvated condition, m. p. 100° and 103° respectively, but from light petroleum in unsolvated yellow prisms, m. p. 127° . It is characterised by a marked retentivity of solvent, which in the case of ether is only removed by fusion in a vacuum. No doubt is felt as to the purity of this material, because the method of fractionation with these two solvents would quickly remove impurities, toxicarol going first to the head and then to the tail of the crystallisations. Its analysis is correct and moreover it gives derivatives in an optically pure condition without recrystallisation. Catalytic hydrogenation gave *l*-dihydrotoxicarol, m. p. 179° , $[\alpha]_D^{20} = -30^\circ$ in benzene, properties which were unchanged after regeneration of the substance from the twice crystallised acetate. Racemisation by sodium acetate-alcohol gave a high yield of *dl*- α -toxicarol, m. p. 219° , giving no colour in the Goodhue test and therefore free from β -toxicarol. The author has obtained similar material by using, on earlier samples of toxicarol, the purification method of Cahn, Phipers, and Boam (*loc. cit.*), whereas they themselves report a melting point of 233° and state that *dl*- α -toxicarol of m. p. 219° contains 4.5% of β -toxicarol. This discrepancy cannot be accounted for. It is noteworthy that Jones [*Ind. Eng. Chem. (Anal. Edit.)*, 1939, **11**, 429], in preparing *dl*- α -toxicarol by the above authors' method, was unable to raise the m. p. above 217° (corr.), though his material still contained a trace of β -toxicarol, and that Clark (*J. Amer. Chem. Soc.*, 1930, **52**, 2461), who first isolated *dl*- α -toxicarol, reported 219° (corr.).

The ethyl acetate mother-liquors from the toxicarol fractionation gave on concentration a small crop of a substance closely simulating toxicarol. By repeated crystallisation from ethyl acetate it was obtained in pale yellow needles melting at 219° . Like *dl*- α -toxicarol, it was optically inactive and phenolic, giving a deep green colour with alcoholic ferric chloride; a mixture, however, gave a well-marked depression of melting point. Their non-identity was further shown by the negative Durham test given by this substance and a methoxyl content of 22.6% as against 15.1% for toxicarol. Elementary analysis, coupled with the methoxyl content, established that the substance has the formula $C_{23}H_{22}O_7$ and is thus isomeric with toxicarol and sumatrol, but has three instead of two methoxyl groups per molecule. Analyses of derivatives described below are in accord with this formula. This substance readily forms solid solutions with *l*- α -toxicarol and it is therefore probable that, coupled with the retention of solvent, it accounts for the high methoxyl contents recorded previously for *l*- α -toxicarol.

It has not been possible in present circumstances to make a complete examination of this substance, but with the material available some of its reactions have been studied. From earlier work a positive Durham test would seem to be specific for the chromenochromanone ring structure *A*, *B*, *C*, and *D* as in toxicarol (I) and sumatrol (II), which is therefore modified or absent in this substance. Moreover it is impossible to formulate a structure of this type containing an additional methoxyl group which yet remains isomeric with toxicarol and sumatrol. Acetylation and benzylation give respectively *O*-monoacetyl and

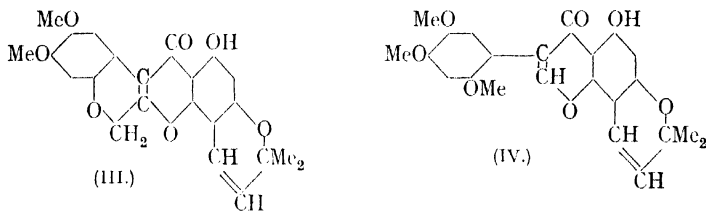


O-monobenzoyl derivatives, giving no colour with ferric chloride, and so establishing the presence of one phenolic hydroxyl group. The strong ferric chloride reaction, coupled

with the insolubility in alkali, suggests the presence of a keto-group ortho to the hydroxyl group, as in toxicarol (I) and sumatrol (II).

Attempts to establish the presence of a keto-group by oximation were unsuccessful, but in the light of the difficulty of oximating toxicarol (George and Robertson, J., 1937, 1535) and of sumatrol (Robertson and Rusby, *ibid.*, p. 497) this cannot be regarded as excluding its presence. If present, it must be incapable of enolisation, as under the conditions used for monoacetylation and monobenzoylation toxicarol gives in addition to mono-derivatives diacetyl- and dibenzoyl-toxicarol through enolisation of the keto-group. This substance is therefore more akin to dehydrotoxicarol (III), which, being incapable of enolisation, gives only a monoacetyl derivative and moreover a negative Durham test. Methylation of this substance with methyl sulphate in potassium carbonate-acetone, giving a *monomethyl ether*, further distinguishes it from toxicarol, which under similar conditions suffers fission of ring C to give an *O*-dimethyl derivative (Cahn, Phipers, and Boam, J., 1938, 734). The stability of this substance to boiling alcoholic sulphuric acid precludes its formulation as an ether of the type of the rotenolone methyl ethers, for these lose methyl alcohol under such conditions (LaForge and Haller, *J. Amer. Chem. Soc.*, 1934, 56, 1620). It does not, however, exclude the presence of an *isopropenyl furan* ring, since, although rotenone is readily isomerised to isorotenone in the presence of acid, sumatrol is recovered unchanged (Robertson and Rusby, *loc. cit.*).

It is not possible from this evidence to establish a formula with any degree of certainty; nevertheless, occurring with toxicarol and sumatrol, the substance is likely to be closely related to them. Therefore as a working hypothesis the *isoflavone* structure (IV) is suggested.



The isolation of this substance in an optically inactive form by direct crystallisation suggests its presence in the resin as such. Preference is therefore given to the presence of a 2:2-dimethyl- Δ^3 -chromen ring as in toxicarol instead of the *isopropenyl furan* ring of sumatrol with its asymmetric carbon atom. The *isoflavone* structure is preferred to that of a flavone, which also is consistent with the data, owing to the closer relationship of the former to toxicarol. Such a formula, if substantiated, is of great biochemical interest as suggesting a link in the biogenesis of toxicarol in the plant. Moreover the possibility is presented of there being a series of *isoflavones* in the plant corresponding to rotenone, etc., and similarly constituted.

dl- α -Toxicarol was isolated by Clark (*Science*, 1930, 71, 396; *J. Amer. Chem. Soc.*, 1930, 52, 2461) from the roots of *Tephrosia toxicaria* previous to its isolation from *D. malaccensis* (Spoon, *De Indische Mercuur*, 1932, 55, 181). Subsequently the isolation of *l*- α -toxicarol from the latter showed that toxicarol was present in the root as the optically active form. It therefore seemed probable that toxicarol was present as *l*- α -toxicarol in *T. toxicaria*. This point has now been elucidated, roots of *T. toxicaria* from both Malaya and British Guiana being used. To facilitate crystallisation, the phenols were separated through their potassium salts and crystallised from ether. After seeding and prolonged refrigeration crude optically active toxicarol separated, which by the ether trituration method (J., 1939, 812) was separated into sumatrol and *l*- α -toxicarol, though the small quantities prevented complete purification. This is the first recorded instance of the occurrence of sumatrol other than in *Derris* root and suggests that it generally accompanies toxicarol. Rotenone was readily separated by crystallisation of the neutral resin from carbon tetrachloride, though its presence in this species has not previously been recorded. It is known, however, to occur in several other species of *Tephrosia*. Subsequent to the completion of this work Castagne (*Contribution a l'Étude Chimique des Légumineuses*

Insecticides du Congo Belge, Brussels, 1938) has reported the isolation of rotenone from this species, but failed to isolate optically active toxicarol.

EXPERIMENTAL.

Microanalyses are by Drs. Weiler and Strauss, Oxford. Methoxyl determinations are by the author, using Clark's semimicro-method (*J. Assoc. Off. Agric. Chem.*, 1932, 15, 136). Melting points were observed in Mason's apparatus (*Chem. and Ind.*, 1925, 577) and are uncorrected.

A number of extractions and fractionations were carried out and this is a typical experiment. The finely ground air-dried root (1320 g.) was extracted to completion with ether in a large Soxhlet apparatus. The extract (1.5 l.), on standing overnight, deposited a gelatinous precipitate (fraction A), which was filtered off and air-dried (25 g.). The filtrate on refrigeration rapidly deposited hard crystalline masses of crude toxicarol. A further quantity could be obtained by concentrating and keeping the filtrate (131 g., fraction B). The ethereal solution was then washed with successive portions of 5% sodium carbonate solution until nothing further was extracted; acidification of these gave fraction C (12 g.). A similar washing with 2% potassium hydroxide solution, acidification, and recovery through ether gave fraction D (14 g.). The ethereal solution was finally extracted with 500 c.c. of 5% potassium hydroxide solution, giving a copious precipitate of the yellow potassium salt of toxicarol, which was recovered by acidification under ether and crystallisation (25 g., added to fraction B). The remaining neutral ethereal extract was washed with acid and water and evaporated, and the residue heated on the steam-bath in a vacuum for 30 mins. to give the neutral resin (35 g.). The examination of this resin will be reported in a forthcoming communication.

Fraction A. The examination of this fraction was reported recently (this vol., p. 309), when it was shown to contain malaccol and sumatrol. Further crystallisation of the mother-liquors has led only to sumatrol.

Fraction C. This acid fraction was obtained by filtration and drying in a vacuum as a red resin, $[\alpha]_D + 18^\circ$ (approx.) in acetone, soluble in ethyl acetate and acetone, but not in hydrocarbon solvents (Found: OMe, 6.5%). With alcoholic ferric chloride it gave an intense red-brown colour and in the Durham test only a very faint green.

Fraction D. This fraction, obtained as a red resin, gave a deep green colour with alcoholic ferric chloride; it had $[\alpha]_D - 31^\circ$ in benzene (*c.* 1.354; *l.* 1) (Found: OMe, 12.6%). A solution in ether on keeping deposited a small crop of yellow dehydro-compounds and the resin on recovery gave a negative Durham test. It could not be obtained crystalline and so was not further examined. This fraction is quite distinct from the phenols soluble in 5% potassium hydroxide solution in giving a soluble potassium salt.

Fraction B. These were bulked from several separations. The crude toxicarol (900 g.) was divided into six lots and crystallised at 0° from 2 vols. of ethyl acetate; further crystallisations separated the material roughly into fractions of differing solubility, which were then subjected to a rigorous fractional crystallisation. After ten crystallisations the head fraction had reached constant specific rotation, $[\gamma]_D - 67^\circ$ in benzene (*c.* 5.00; *l.* 1),* and a series of head fractions with this rotation were taken off (250 g., fraction E). The ethyl acetate solutions were then bulked and concentrated; no further toxicarol separated, but a small crop of material, obviously not toxicarol though with m. p. $100-105^\circ$, was obtained (2 g., fraction F). The ethyl acetate solution was evaporated, and the resin dissolved in ether (2 l.) and washed with 500 c.c. of 2% potassium hydroxide solution. After drying, the ethereal solution was refrigerated, crude toxicarol separating rapidly. A further crop was obtained by concentration (302 g., fraction G). The filtrate has not so far deposited any further crystals.

Fraction E. This was fractionated by mechanical shaking with 10 vols. of ether for 30 mins., filtration, and concentration of the filtrate to one-third of its bulk for crystallisation. The insoluble residue was re-treated with ether (cf. *J.*, 1939, 812) as follows:

240 g., $[\alpha]_D - 67^\circ$	
Filtrate	Residue
136 g., $[\alpha]_D - 67^\circ$, OMe 15.1%	80 g.
Filtrate	
42 g., $[\alpha]_D - 67^\circ$, OMe 15.3%	Residue
	25 g., $[\alpha]_D - 67^\circ$

* All samples of toxicarol, unless otherwise stated, were fused in a vacuum at 110° for 30 mins. before analysis and determination of $[\alpha]_D$ in 5% benzene solution.

The major most soluble fraction was therefore unchanged and is undoubtedly pure *l*- α -toxicarol. As a check a 20 g. portion was re-treated with ether, giving from the filtrate a crop (11.5 g., $[\alpha]_D^{20} - 67.4^\circ$, OMe 15.2%), and a residue (6.0 g., $[\alpha]_D^{20} - 67.4^\circ$, OMe 15.0%). The material was therefore homogeneous.

l- α -Toxicarol crystallised from ether in bright yellow laths, m. p. 100° with evolution of solvent. The crystals contained ether, which was only slowly lost on drying but did not correspond to any simple solvate [Found: OMe (air-dried material), 17.0; (material dried in a vacuum at 75° for 6 hrs.), 16.3; (material fused in a vacuum) C, 67.4; H, 5.6; OMe, 15.1. Calc. for $C_{23}H_{22}O_7$: C, 67.3; H, 5.4; OMe, 15.1%]. From ethyl acetate-alcohol it crystallised in a similarly solvated condition in bright yellow laths, m. p. 103° [Found: OMe (material dried in a vacuum at 75° for 6 hrs.), 16.0%]. However, from light petroleum (b. p. $60-80^\circ$) *l*- α -toxicarol crystallised in unsolvated yellow prisms, m. p. 127° , $[\alpha]_D^{20} - 67^\circ$ in benzene, $+37^\circ$ in chloroform, and $+61^\circ$ in acetone (c, 5.00; l, 1) [Found: OMe (air-dried material), 15.1%]. It gave no colour in the Goodhue test (*J. Assoc. Off. Agric. Chem.*, 1936, 19, 118).

l-Dihydrotoxicarol.—Reduction of *l*- α -toxicarol as described previously (J., 1939, 815) gave *l*-dihydrotoxicarol in pale yellow needles, m. p. 179° , $[\alpha]_D^{20} - 30^\circ$ in benzene (c, 5.00; l, 1) (Found: OMe, 15.1. Calc. for $C_{23}H_{24}O_7$: OMe, 15.0%). Acetylation (*loc. cit.*) gave the *O*-acetyl derivative in colourless needles, m. p. 184° , $[\alpha]_D^{20} - 59^\circ$ in acetone (c, 5.06; l, 1) (Found: OMe, 13.6. Calc. for $C_{25}H_{26}O_8$: OMe, 13.6%). Calm, Phipers, and Boam (J., 1938, 534) have stated that this compound can be hydrolysed by boiling with 5% alcoholic hydrochloric acid for 30 mins.; repetition of this, however, led to mainly unhydrolysed material. Finally the compound was refluxed with 5% alcoholic sulphuric acid for 6 hrs.; on cooling, *l*-dihydrotoxicarol of the same m. p. and rotation as above separated, unaltered by further crystallisation.

dl- α -Toxicarol.—*l*- α -Toxicarol (5 g.) and sodium acetate (10 g., anhydrous) were refluxed for 2 hrs. in ethyl alcohol (100 c.c.). The liquid was filtered hot, and the solid washed with alcohol and hot water, to give *dl*- α -toxicarol (4.0 g.), m. p. 219° . The m. p. was not raised by crystallisation from acetic acid (Found: OMe, 15.0%). The product gave no colour in the Goodhue test and was therefore free from β -toxicarol.

Fraction F.—After four crystallisations from ethyl acetate, in which it was sparingly soluble, this fraction gave a substance in pale yellow needles, m. p. 219° , $\alpha_D \pm 0^\circ$ in chloroform. In the Durham test it gave no colour, but with alcoholic ferric chloride a deep green colour and with concentrated sulphuric acid a deep orange non-fluorescent solution were obtained. Before analysis this and its derivatives were dried in a vacuum at 100° for 1 hour (Found: C, 67.3; H, 5.45; OMe, 22.6. $C_{23}H_{22}O_7$ requires C, 67.3; H, 5.4; 3OMe, 22.7%).

O-Monoacetyl derivative. The substance (98 mg.) was refluxed in acetic anhydride (1 c.c.) and pyridine (0.5 c.c.) for 1 hour and poured into water. The precipitate was crystallised from alcohol to give the monoacetate in colourless needles, m. p. 210° (Found: C, 66.1; H, 5.25; OMe, 20.65. $C_{25}H_{24}O_8$ requires C, 66.35; H, 5.3; OMe, 20.6%). It gave no colour with alcoholic ferric chloride.

O-Monobenzoyl derivative. The substance (100 mg.) was refluxed with benzoyl chloride (0.2 c.c.) and pyridine (1 c.c.) for 1 hour. After decomposition with water the precipitate was dissolved in chloroform-alcohol and crystallised by evaporation of the chloroform. The monobenzoate was obtained in colourless prisms, giving no colour with ferric chloride; m. p. 193° (Found: C, 68.7; H, 5.0; OMe, 18.3. $C_{30}H_{26}O_8$ requires C, 69.9; H, 5.1; OMe, 18.1%).

O-Monomethyl derivative. The substance (100 mg.), methyl sulphate (0.5 c.c.), and potassium carbonate (200 mg.) were refluxed for 6 hours in acetone (15 c.c.). Next day the ferric chloride reaction was still positive, so methyl sulphate (0.5 c.c.) and potassium carbonate (200 mg.) were added and refluxing continued for another 6 hours; the ferric reaction was then negative. By pouring into water and crystallisation from benzene-light petroleum the ether was obtained in colourless prisms (82 mg.), m. p. 178° (Found: C, 66.4; H, 5.4; OMe, 28.0. $C_{24}H_{24}O_7$ requires C, 67.9; H, 5.7; OMe, 29.2%).

Fraction G.—From the analysis ($[\alpha]_D^{20} - 84^\circ$; OMe, 15.2%) this fraction appeared to consist only of a mixture of toxicarol and sumatrol. After two crystallisations from ethyl acetate-alcohol it was subjected to ether trituration (as described for fraction E), giving a residue (30 g.) of nearly pure sumatrol, m. p. 190° . The ethereal filtrates were concentrated, and the crops recrystallised from ethyl acetate-alcohol to give pure toxicarol identical with that prepared above.

Tephrosia toxicaria.—The ground root (1500 g.) (from Malaya) was extracted with ethyl acetate in a large Soxhlet apparatus. The pale red extract was evaporated, and the resin treated

with two 500 c.c. portions of ether, filtering into a separating-funnel. This extract was washed with successive portions of 5% potassium hydroxide solution saturated with sodium chloride. Only those portions were retained that gave a yellow precipitate of toxicarol salt. The neutral ethereal layer was dried and evaporated, and the resin (29 g.) dissolved in carbon tetrachloride (150 c.c.). After seeding and refrigeration, rotenone-carbon tetrachloride solvate (6.0 g.) separated. Recrystallisation from carbon tetrachloride and then from alcohol gave rotenone (3.2 g.; yield, 0.2%, calculated on the weight of root), m. p. 163.5°, undepressed on admixture with an authentic specimen. The alkaline extracts, containing yellow potassium salt, were acidified under ether, and the extract washed, dried, and concentrated to 200 c.c. On standing overnight, dehydro-compounds (0.3 g.) separated; then, on refrigeration over a period of 4 months, the filtrate deposited crude *l*- α -toxicarol (9.2 g.). This was crystallised once from ethyl acetate-alcohol and then triturated with ether. The residue on crystallisation from ethyl acetate gave a small crop of dehydrotoxicarol and on addition of alcohol to the filtrate a crop of sumatrol (0.6 g.) admixed with dehydro-compounds. After separation by hand the sumatrol had m. p. 182°, undepressed on admixture with an authentic specimen. The ether-soluble fraction on concentration gave *l*- α -toxicarol (3.0 g.). Crystallised once from ethyl acetate-alcohol, it had m. p. 98°, $[\alpha]_D^{25}$ -- 77° in benzene. The m. p. was undepressed by an authentic specimen, but this preparation evidently still contained sumatrol.

Extraction of a sample of *T. toxicaria* from British Guiana gave similar results, both rotenone and toxicarol being isolated.

I am indebted to the Directors, the Departments of Agriculture, Malaya, and British Guiana, for the supply of root, and to the Ministry of Agriculture and the Colonial Development Fund for grants which have made this work possible.

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THE CHEMICAL
EVALUATION OF PYRETHRUM FLOWERS
(*CHRYSANTHEMUM CINERARIIFOLIUM*)

THE EXTRACTION OF THE FLOWERS FOR ANALYSIS AND
THE PREPARATION OF COLOURLESS CONCENTRATES OF
THE PYRETHRINS

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(With One Text-figure)

INTRODUCTION

IN the determination of the pyrethrins, the insecticidal principles of pyrethrum flowers, it is customary to extract the ground flowers with low-boiling petroleum ether. This solvent is used because of its selective action, extracting the active principles with a minimum of extraneous matter. Ripert (1934), however, has suggested that owing to the protective action of oxyacids, formed particularly on storage and insoluble in petroleum ether, the pyrethrins may not be completely removed by this solvent, and has found (1936) a chloroform extract of the flowers made after extraction with petroleum ether to be highly toxic to house-flies. Gnadinger (1936) points out the possibility of the extraction by ether of changed pyrethrins, reacting in the analytical methods as true pyrethrins, but of lower toxicities. The position has been discussed, from the chemical point of view, in a preliminary way by Martin (1938). The present communication gives the results of biological trials carried out on ether extracts of the flowers made after preliminary extraction with petroleum ether.

We have shown (Martin & Potter, 1937) that if the powdered flowers are intimately mixed with decolorizing charcoal, and then extracted in a Soxhlet apparatus with petroleum ether, a colourless extract results. Further information as to the nature and pyrethrin contents of such extracts is now given. The work was undertaken because of the need, for a separate investigation, of a concentrate of the pyrethrins, and it was considered that a colourless extract, made by the extraction of flowers admixed with charcoal, would provide a suitable starting material for this work.

Table 2. *Toxicities to Aphis rumicis of petroleum ether and ether after petroleum-ether extracts of pyrethrum flowers*

% of flowers	% of resin	% pyrethrin I	% mortality allowing for control	% S.E.* ±
Petroleum-ether extraction				
0.23	0.0108	0.0012	100	—
0.15	0.0072	0.0009	100	—
0.075	0.0036	0.0004	75.8	3.7
0.038	0.0018	0.0002	35.1	3.3
Ether after petroleum-ether extraction				
1.50	0.0458	0.0006	70.3	5.7
1.13	0.0344	0.0005	62.6	4.1
0.75	0.0229	0.0003	26.3	14.5
0.45	0.0138	0.0002	32.9	12.2
0.15	0.0046	0.0001	14.7	6.3
Control, alcohol-saponin				
—	—	—	10.0	—

* Calculated on percentage mortalities before allowing for control.

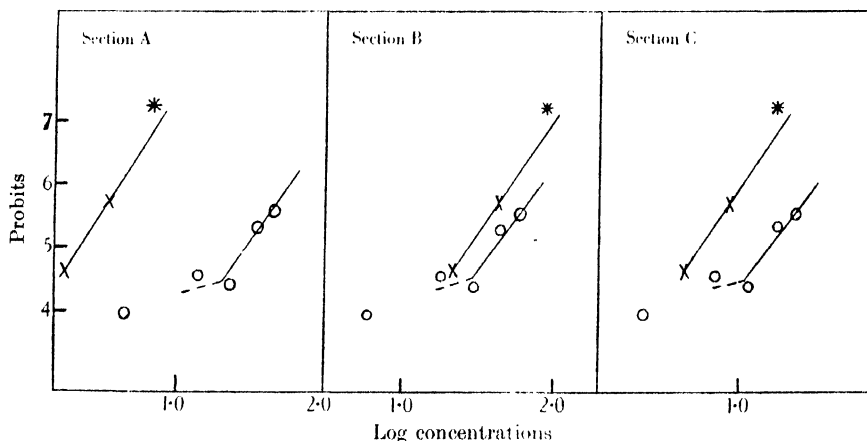


Fig. 1. Probits plotted against the log concentrations of the resins extracted by petroleum ether and subsequent ether extraction (Section A), of pyrethrin I (Section B) and of total pyrethrins (Section C).

x Petroleum-ether extraction. o Subsequent ether extraction.
 * Calculated value for 100 % mortality.

petroleum ether is only just outside the experimental error of the Seil method. A definite toxic effect to *Aphis rumicis* was obtained when the ether-extracted resin was tested at high concentrations, but judging from the concentrations required for 50 % mortality of the insects (Fig. 1), the original flowers were of the order of 18 times more toxic than the same flowers after petroleum-ether extraction. The ether-extracted resin was slightly less toxic, by comparison with the petroleum-

ether-extracted resin, than its apparent pyrethrin I content would have indicated, and was definitely overvalued by its apparent total pyrethrin content. It is probable that the value of 0.12 % is an overestimate of the amount of true pyrethrin II occurring in the ether-extracted resin. Extraction by percolation with petroleum ether for 3 hr. has thus accounted for something like 95 % of the biological activity of the flowers tested. In view of its selective action, this solvent should therefore be retained for the extraction of the flowers for analysis, a minimum period of 8 hr. being employed.

The preparation of colourless concentrates of the pyrethrins

Comparison of petroleum ether and chloroform extracts of the flowers, with and without admixture with charcoal. The flowers used were taken from an experimental bed of pyrethrum at Harpenden, and were characterized by a high content of pyrethrin I in comparison with that of II. A portion of 37.5 g. was exhaustively extracted, in subdued light, in a Soxhlet apparatus with low-boiling petroleum ether. Further portions of 37.5 g. were intimately mixed with 2.5 and 5.0 g. of decolorizing charcoal, freshly received from the manufacturers, and similarly extracted with petroleum ether. The extracts were made up to 250 ml. and 100 ml. aliquots used for the tests.

Free acids were removed by the addition of water to the petroleum-ether solution in a separating funnel and titration with 0.02 *N* alkali to phenolphthalein. There was a distinct tendency for the formation of an emulsion in the case of the flowers extracted directly with petroleum ether, but not where the flowers had been admixed with charcoal. The petroleum-ether solutions were washed, dried, the solvent removed and the resins recovered and weighed.

Saponification was carried out with 0.5 *N* methyl alcoholic potash, the alcohol was removed by warming under reduced pressure, and the pyrethrins determined by the method of Seil (1934) using 1-2 ml. of normal acid for the acidification of the filtrate from the barium precipitation. The results are given in Table 3.

Further tests were carried out, using the same flowers but with chloroform as solvent. In this case, it was found necessary to increase the (fresh) charcoal content of the mixture to at least 45 % in order to obtain an almost colourless extract. The results are given in Table 4.

The comparison of the pyrethrin contents of the flowers given by direct petroleum-ether and chloroform extraction is of interest. Chloroform extraction has resulted in only a slightly higher value for pyrethrin I,

but in an appreciably higher figure for pyrethrin II. This effect has been noted before (Martin, 1938) when the pyrethrin contents of flowers given by petroleum ether and ether extraction were compared. It is unlikely that petroleum ether exerts a preferential extraction of pyrethrin I from the flowers. In view of the additional fatty material extracted by ether or chloroform, it is probable that the resulting values for pyrethrin II are overestimates of this constituent, a proportion of fatty acid, non-volatile in steam and soluble in ether being determined with the dicarboxylic acid.

Table 3. *Comparison of petroleum-ether extracts of the flowers, with and without charcoal*

	No charcoal	Charcoal, % of mixture	
		6.3	11.8
Colour of extract	Deeply coloured	Very pale yellow	Colourless
Resin % of flowers	3.6	2.9	2.0
Ml. of 0.02 N alkali to neutralize free acids	8.5	3	2
Pyrethrin I % of flowers	0.85	0.87	0.74
Pyrethrin II % of flowers	0.31	0.26	0.17
Total pyrethrins % of resin	32	39	45

Table 4. *Comparison of chloroform extracts of the flowers, with and without charcoal*

	No charcoal	Charcoal,
		45 % of mixture
Colour of extract	Deeply coloured	Pale yellow
Ml. of 0.02 N alkali to neutralize free acids	25	8
Resin % of flowers	8.27	4.75
Pyrethrin I % of flowers	0.88	0.85
Pyrethrin II % of flowers	0.52	0.39
Total pyrethrins % of resin	17	26

It is clear from Table 3 that a colourless extract obtained by the use of charcoal containing 45 % of pyrethrins, and a reduced content of substances with emulsifying properties should be readily amenable to further purification in the preparation of pyrethrin concentrates. Further work on such extracts was therefore carried out, based upon the methods used by LaForge & Haller (1935).

Preparation of pyrethrin concentrates. In a preliminary test, 50 g. of flowers mixed with charcoal was extracted with petroleum ether. In this case, charcoal which had been standing in the laboratory for a considerable time was used, and it was found necessary to incorporate approximately 35 % of charcoal in the mixture before a colourless extract was obtained. Free acids were removed and the colourless resin, dissolved

in acetic acid containing 10 % of water, was cooled to 0° C. The fatty material separating was removed, and an oil recovered by dilution of the filtrate with water and extraction with petroleum ether.

The total pyrethrin content of the colourless oil, determined by the Seil method, was 78 %, made up of 59 % of I, and 19 % of II.

A second experiment was carried out, in which 700 g. of flowers were mixed with 420 g. of charcoal (old) and extracted with petroleum ether. A pale yellow extract resulted. On concentration and refrigeration for some days, white crystals separated. These were filtered off and the free acids removed from the filtrate with dilute alkali. The resin (14 g.) was recovered and dissolved, with warming, in glacial acetic acid. On cooling, further crystalline material separated. This was filtered off and washed with acetic acid; 10 % of water was then added to the filtrate and the solution cooled to 0° C. Fatty material separating was filtered off. The acid solution was then extracted with two volumes of petroleum ether, the petroleum-ether solution washed four times with acetic acid containing 10 % of water, and then repeatedly with water. The solution was dried over sodium sulphate and the solvent removed, to yield 4.2 g. of a pale yellow oil.

This showed by the Seil method a total pyrethrin content of 78 %, made up of 65 % of I and 13 % of II.

The oil (2 g.) was dissolved in petroleum ether and slowly run through a column of charcoal ($3 \times 1\frac{1}{4}$ in.) previously wetted with the solvent. The charcoal was washed repeatedly by percolation with petroleum ether. On removal of the solvent from the percolate, 0.1 g. in all of a colourless aromatic oil resulted. The charcoal was then percolated for two periods of 6 hr. each with ether. The first extraction yielded 1.33 g. and the second 0.20 g. of a colourless oil. The charcoal, after mixing, was percolated for $7\frac{1}{2}$ hr. with chloroform to give 0.25 g. of a yellow resin.

Of the oil taken, 93 % was thus accounted for. The colourless oil (1.33 g.) yielded by the first ether extraction, on analysis by the Seil method, showed an average total pyrethrin content of 93 % (made up of 81.7, 81.0 % of I and 12.1, 11.3 % of II in duplicate analyses).

The flowers originally taken showed a ratio of pyrethrin I to pyrethrin II of 2.7. This has been changed in the final concentrate to a value of 7. Such a concentrate should be of value for the isolation of pyrethrin I by distillation.

It should be stressed that the quality of the charcoal used plays an important part in the amount needed to obtain a colourless extract. This is clearly seen when the amount used in the preparation of the

colourless extract by petroleum-ether extraction (Table 3) is compared with those that were required in obtaining extracts for the preparation of the pyrethrin concentrates. It was thought possible at one stage that the incorporation of charcoal with the flowers would assist the analytical process, but it was found that the variable nature of the decolorizing charcoal available made the assessment of the amount to be used a matter of some difficulty.

There has been evidence in the work that pyrethrin II tends to be retained by the charcoal more tenaciously than is pyrethrin I, and this fact may be capable of utilization in a further separation of the active principles.

SUMMARY

1. Biological trials have been carried out to determine the efficacy of petroleum ether as solvent for the extraction of pyrethrum flowers for analysis. 95 % of the toxic material was extracted from flowers one year old after only 3 hr. percolation. An extraction period of 8 hr. with petroleum ether is suggested.

2. A method of preparing colourless extracts of pyrethrum and analytical data for such extracts are given. They are shown to be of value for the preparation of concentrates of the pyrethrins. The preparation of a colourless concentrate containing 93 % of total pyrethrins, as determined by a modified Seil method, is described.

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A LABORATORY SPRAYING APPARATUS AND TECHNIQUE FOR INVESTIGATING THE ACTION OF CONTACT INSECTICIDES. WITH SOME NOTES ON SUITABLE TEST INSECTS

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(With Plate 8 and 5 Text-figures)

To ascertain whether any substance is likely to be useful as a contact insecticide two separate investigations have to be made: a laboratory examination of the effect of the substance on insects, and a study of it in the field under the conditions in which it is to be used in practice. In order to ascertain how a contact insecticide can be applied to the best advantage, it is necessary to make a detailed study of its effect both qualitatively and quantitatively, and this can be most satisfactorily carried out under the controlled conditions only to be obtained in the laboratory.

Laboratory studies on contact insecticides in a fluid medium can be classified under three heads: (1) The discovery of new substances which possess insecticidal activity, and a general investigation into their possibilities. (2) The detailed study of any given substance to determine its physiological action and its effect on particular species of insects. (3) The study of the effect of varying the medium in which the toxic substance is applied, of the methods of application and of factors extraneous to the toxic substance.

The first requisite for the study of these three problems is a method of applying the toxic liquids in equal doses to large numbers of individual insects of a wide variety of species. The method should also be such that the individual doses administered may range from small to large. The second requisite is to have available a wide range of species with representatives from all the important orders of insects. It should be possible to rear some, at any rate, of these species so that large numbers are continuously available; for this purpose, species with a short life cycle and no seasonal rhythm are most suitable.

In the present work most of the time was devoted to the method of applying the dose of insecticide, but some preliminary work was also done on the selection and rearing of suitable test insects. Once a suitable method of application of the insecticide was found, the extent to which results obtained with any given species and instar are generally applicable, depends on the degree to which specificity of effect occurs, and this will probably vary both with the insecticide and the insect. The information on this subject is scanty, and it is desirable that more data should be gained on the subject of specificity as a preliminary to the selection of a range of species as test insects. In the meantime it has to be assumed that an insecticide which fails to kill the adult of one or two species of insect which have not so far been found highly resistant, is of little value as an insecticide.

APPARATUS AND METHODS AT PRESENT AVAILABLE

Tattersfield (1939) reviewed biological methods of testing insecticides, including a brief description and discussion of the laboratory methods of testing liquid contact insecticides so far used. The means of applying liquids to the integument of an insect can be divided into

three classes: (1) spraying, (2) dipping, (3) the use of the micropipette. The dipping and micropipette methods were discarded because they were too far removed from practice and could give no data on some of the problems outlined in the preceding paragraphs. The dipping method was thought to have the further disadvantage that stomach poison and a contact poison effect might be indistinguishable. The micropipette method becomes extremely laborious if the large number of insects required for the accumulation of data suitable for accurate statistical analysis are used.

The desirable attributes of a laboratory spraying apparatus are: (1) that a given quantity can repeatedly be deposited on the sprayed area, so that equal doses may be applied for purposes of comparison; (2) that the spray should be evenly deposited over the sprayed surface so that insects distributed over that area may each receive an equal dose; (3) that a sufficiently large area should be evenly coated, so that insects of various sizes and rates of locomotion may be used; (4) that it should be possible to vary the amount of spray deposited within wide limits from a very light deposit to a very heavy deposit; this is most necessary where media which are themselves toxic are used; (5) that the apparatus should be suitable for use with a wide variety of fluids.

Omitting the lethal chamber methods employed for testing fly sprays, the spraying methods that have been used can be put under four heads: (1) the Tattersfield method; (2) the Campbell turn-table method; (3) the method of O'Kane *et al.*; (4) the method of Hartzell & Wilcoxon.

The Tattersfield method (Tattersfield & Morris, 1924; Tattersfield, 1934) has been successfully used for the qualitative and quantitative evaluation of a large number of insecticidal substances and is the only spraying apparatus for which full data of its physical and biological performance have been published. It has the advantage of simplicity and accuracy. Its disadvantages are that the area that can be relatively evenly coated with spray is small so that only small slow-moving insects or the inactive stages of insects may be used, and it is difficult to reduce the weight of deposits beyond a certain minimum value.

The Campbell turn-table method (Jones *et al.* 1935; Campbell & Sullivan, 1938) appears to have been widely used, but there are very few data on its performance, both physical and biological. On a priori grounds, it appears likely that the spray is evenly deposited over the sprayed area, but the total amount deposited after each spraying may not be constant, and it also appears that it would be difficult if not impossible to apply other than light deposits. Further, the insects must be rendered inactive before treatment, since they necessarily have a long period of exposure, during which they might crawl off the sprayed area if not previously inactivated.

The method of O'Kane *et al.* (1930) does not appear to be sufficiently flexible and quantitative. The method of Hartzell & Wilcoxon (1932) does not appear to fall into the category of quantitative laboratory testing; it has more the nature of an accurate preliminary field trial, and presumably was not designed to answer the questions that are raised here.

Evolution of apparatus. Sources of error in replication and distribution of deposit

When it became necessary to devise a laboratory spraying apparatus suitable for testing contact insecticides on a wide range of species of insects, the Tattersfield apparatus was taken as a starting point, since this was the only apparatus the performance of which had been fully investigated and published. The outstanding disadvantage of this apparatus was

the small area that could be covered without a severe falling off of the amount of spray deposited.

By using a variety of atomizing nozzles to produce the jet of spray it was found that all of these, when applied directly to a surface, give a very much heavier deposit in the centre falling off rapidly to the outside of the sprayed area. This difficulty could be overcome by breaking up the cone of spray after it issued from the atomizing nozzle by inducing turbulence, by spraying through a tube. It was found that a number of factors influences the degrees of turbulence and thus the evenness of the deposit, the most important being: (1) the diameter of the tube; for tubes of a given length the smaller the diameter the greater the turbulence: (2) the length of the tube; the length of tube necessary to obtain sufficient turbulence to produce an even deposit varied inversely with the diameter of the tube: (3) the nature of the apparatus at the top and bottom; closing the openings at the top and bottom of the tube both tended to increase turbulence and produce a more even deposit: (4) volume and rate of flow of air; increased volume and rate of flow of air produced by raising the air pressure cause greater turbulence under given conditions.

With these facts in mind an apparatus was constructed as shown in the photograph (Pl. 8*a*). The apparatus consisted of a square tube of glass and metal, the upper narrower part of the tube acting as a mixing tower in which turbulence took place and a lower wider part acting as a settling chamber in which the liquid droplets settled out on the spray plate. An aerograph M.P. paint gun was used to atomize the spray fluid. This instrument was closed at the bottom when spraying was taking place and was closed at the top except for four small exit holes to relieve the pressure. It was constructed at the Imperial College of Science and Technology and used there for a number of experiments which have since been published (Potter, 1938; Potter & Musgrave, 1940; Callaway & Musgrave, 1940). This was used with insecticides in an oil medium alone; the apparatus was found to give a fairly even deposit over a 6 in. plate, but the total deposit varied considerably from one spraying to the next, and throughout the work the total deposit had to be checked by weighing. It was further necessary to atomize large amounts of material in order to obtain sufficient deposit on the spray plate, a very high proportion of material being thrown out on to the sides of the spray tower. At this point the work was taken up at Rothamsted where more attention was given to aqueous fluids than to sprays with an oil base.

Using the type of apparatus shown in Pl. 8 *a* and *b* the variation in total deposit in a series of spraying trials was normally from 10 to 20 % and might be as high as 30 %. The causes for this variation were obscure and a large number of experiments were made to elucidate the difficulty, numerous different atomizing nozzles and a variety of lengths and shapes of tube which were closed both top and bottom in varying degrees being used. A short series of experiments was also made on what were considered to be the most likely causes of variation, i.e. (1) effect of air currents, (2) effect of temperature and humidity, (3) electrostatic effects.

(1) *Effect of air currents.*

Although an even distribution can be made by inducing turbulence, it seems as if this is also liable to introduce errors in the replication of the amount of deposit from one spraying to the next. A possible reason for this is that the critical velocity at which an air stream becomes turbulent is not constant even under relatively constant conditions. It is therefore

probable that turbulence commences at different depths in the spraying tower in different sprayings, and since the higher up in the spray tower turbulence starts, the more material is thrown out on the sides of the vessel and the less reaches the bottom, it can easily be understood that this instability of the point at which turbulence commences is a likely source of error. No means have been found of verifying this experimentally, but all the general evidence gathered from over 2500 measurements by weighing of deposits points to the fact that where there is a great deal of turbulence there is a greater liability of error.

It appears that evenness of air pressure over the sprayed area is one essential in the production of an even deposit. An apparatus was set up to produce an even deposit and then a separate jet of air alone was directed on the plate upon which the deposit was falling; at whatever point the air jet impinged on the plate a very heavy deposit was formed.

When these points had been established, all efforts were directed to evening out the air pressure over the sprayed area with the minimum of turbulence. This meant, among other things, working at considerably lower air pressures than had previously been used. It was also found that an adjustable gap at the bottom of the spray tower was a convenient method of regulating the air currents. This was obtained by having a circular plate of the same area as the bottom of the tower mounted in such a way that the gap between it and the spray tower could be varied and at the same time accurately adjusted. As the gap was increased, so the amount of turbulence decreased and the deposit tended to become heavier at the centre. By adjusting the gap to the maximum which could be used to produce an even deposit, the latter can be obtained with the minimum of turbulence.

(2) *Effect of temperature and humidity.*

Using aqueous sprays, little difference could be detected for temperature changes of 1–2° C. Differences above 5° C. appear to affect the deposit, probably due to the alteration produced in the saturation deficiency.

With the large surface area produced by atomization, evaporation effects must be important. That this is so is shown by using a volatile liquid such as alcohol or acetone when only a very small proportion of the material reaches the spray plate, but it does not appear that a change of 5 % in the relative humidity has any detectable effect when water is used as the medium at normal laboratory temperatures. No experiments were made to determine the separate effects of humidity and temperature, and it frequently happens that a large change in the relative humidity is accompanied by a considerable change of temperature, so that these effects must be considered together. Small changes in either do not appear to be important.

(3) *Effect of electrostatic change.*

It is known that when liquids are atomized the particles may acquire an electric charge. The magnitude and sign of this charge varies with the liquid and the conditions. This matter as it affects spray deposit has been studied by Wampler & Hoskins (1939). Preliminary experiments were made to find out if this charging of the atomized particles was likely to be a cause of serious variations in deposit under the experimental conditions described.

First experiments were done by spraying liquids on to a metal target and conducting the charge off the target either to a gold-leaf electroscope or to a valve electrometer. Distilled water was first used and a much smaller charge was produced when the target was sprayed

through a glass spray tower than when it was sprayed directly. This was almost certainly due to the deposition on the sides of the tower, much less liquid reaching the target when it is sprayed through the tower. It was then found that drops of liquid dripping from the glass tower were highly charged, each drop causing a violent deflexion on the valve electrometer. When distilled water was sprayed through an earthed metal tower, the particles reaching the target were still charged, but the drops from the tower were not charged. When water containing traces of saponin was sprayed the charge was doubled and a very large charge was obtained with a very dilute emulsion of xylol in water. Water containing a little dissolved electrolyte gave no detectable charge.

It was clear from these experiments that, at least under certain conditions, electric charges might be a source of variation; more particularly, it appeared that a glass tower was liable to become charged and provide an electric field of varying intensity through which the atomized droplets had to pass. It was therefore considered that it would be preferable to use an earthed metal tower which would not only not become charged itself, but would provide a faraday screen, thus giving a uniform field through which the particles passed. When an earthed metal tower was used, errors of replication were sensibly reduced. This cannot with certainty be ascribed to a reduction of electrical effects, since the metal tower used, although similar was not exactly the same shape as the glass one which it replaced; it is, however, very likely that the slight difference in shape was immaterial, since towers of various shapes had previously been tried without greatly affecting the errors.

Further investigation into electrostatic effects was not made, since to do so would mean a long and involved study, which although of value might not be directly applicable to the work in hand. The work on electric charge was only carried out after a considerable amount of experimental work on the apparatus had already been done, the instrument shown in the photograph (Pl. 8 *b*) being used for a large amount of insecticide testing. This apparatus gave a good distribution, and errors of replication of the deposit were usually within 15 %. The apparatus finally decided upon as being the most suitable is described in the next paragraph.

Construction of final apparatus

This consists of a reservoir and a specially designed atomizing nozzle mounted on a small circular plate carried on three bars, each at an angle of 120° with the other. The end of each bar rests on the top of the metal tower through which the spray is directed on to the spray plate or dish. The spray plate or dish is carried on a table formed by the bottom plate which has a universal adjustment, the whole being mounted on a wooden stand. (See Pl. 8 *c*.)

The atomizing nozzle.

Numerous types of atomizing nozzles have been tried, including paint spray guns (Aerograph type M.P.), air brushes (Aerograph), water spray nozzles (Aerograph type F.N. with various sizes of nozzles and cap), specially constructed nozzles by F. W. Pellant, similar in type to those used on the Tattersfield-Morris apparatus, and a variety of scent-spray nozzles and nozzles constructed in the laboratory. It was found that all these nozzles were capable of fairly good replication of deposit when sprayed directly on to a surface so that at least 20 % of the material in the reservoir was collected and no modification of the direct cone of spray was made, the difference between the highest and lowest readings ranging from 4 to 10 %. Under these conditions, however, there was invariably a great falling off in amount of deposit from the centre to the outside. When any attempt was made to modify the cone of spray to produce a more even distribution, the errors in replication of deposit were considerably increased in all the nozzles, the difference between the highest and lowest deposits for a series being from 15 to 30 %. The degree of atomization with most of the nozzles used was adjusted by means of a needle valve in the liquid feed; in practice this valve tended to produce clogging, thus markedly

altering the weight of deposit and taking up considerable time for adjustment. A further disadvantage in all the nozzles available is that the liquid jet is almost invariably not quite centrally placed in the air jet, and no means are available whereby the jet may be centred in order to distribute the liquid evenly over the cone of spray. Further, there are no means of adjusting vertically the position of the tip of the liquid jet in relation to the tip of the air jet, an adjustment that markedly affects the degree of atomization.

Accordingly an atomizing nozzle was constructed which is as simple as possible, but has the two adjustments last mentioned (see Pl. 8 *d*, and the scale diagram Text-fig. 1). It consists essentially of two parts: (a) a top circular brass plate (*TP*) 2 in. diam. and $\frac{1}{4}$ in. thick, on which is mounted a liquid jet (*LJ*) with an adjusting nut (*AN*) working against a friction washer (*FW*) for moving the jet up and down, the liquid feed tube (*LFT*) and the liquid reservoir (*R*); (b) a bottom circular brass plate (*BP*) 2 in. diam. and $\frac{3}{16}$ in. thick joined by a narrow brass neck to a mounting plate (*MP*) in which is bored the air jet (*AJ*). The tube to the compressed air supply (*AT*) is led into the brass neck.

When assembled, the top plate is screwed to the bottom plate by means of three adjusting screws (*AS*) and there is a compressible rubber washer (*W*) in between. The two plates, screws and washer together form a universal joint. The liquid feed tube must form an airtight joint in the top plate, but at the same time be capable of movement up and down; this is done by having a stuffing box (*SB*), through which the tube passes, mounted on the top plate.

In order to adjust the nozzle for working, the liquid jet is first roughly centralized by means of the three adjusting screws (*AS*); it is then pushed through the liquid jet as far as it will go and adjusted to the required depth by tightening the adjusting nut (*AN*); finally, it is accurately centred by means of the adjusting screws (*AS*). The following are the essential dimensions of the various parts: air-jet orifice, 0.0635 in.; liquid jet orifice, 0.0135 in.; internal diameter of compressed air tube $\frac{1}{16}$ in.; thickness of rubber washer, $\frac{5}{32}$ in.; internal diameter of liquid feed, $\frac{1}{16}$ in.; capacity of reservoir, 10 c.c.

This nozzle worked well in practice; the accurate centring of the liquid jet in the air jet helps to obtain a symmetrical and even distribution and the absence of any form of needle valve in the liquid feed reduces the risk of blocking. The normal function of the needle valve is to alter the degree of atomization, but this purpose can be achieved in the nozzle described by altering the air pressure and by adjusting the position of the tip of the liquid jet relative to the orifice of the air jet.

Atomizing nozzle mounting (Pl. 8 *d*).

The atomizing nozzle is mounted on a plate of the same diameter as the mounting plate on the nozzle, i.e. 2½ in. This plate carries three bars extending out radially, each at an angle of 120° with the other. The bars are 3½ in. long measured from the circumference of the plate, $\frac{1}{2}$ in. wide and $\frac{3}{16}$ in. thick. 2 in. from the end they carry adjusting screws which work against the inside of the spray tower, by means of which the nozzle is centred in the tower. $\frac{5}{16}$ in. from the end are adjusting screws which work against the top of the tower and by means of these, the cone of spray can finally be adjusted to spray down the centre of the tower.

The spray tower.

This consists of a metal tube which is earthed. In the apparatus described this is of galvanized iron $\frac{1}{32}$ in. thick, but it would be preferable if constructed in some form of thicker rustless material. The tube is 26 in. long, both the top and the bottom having a 1 in. flange. The internal diameter of the top is 6½ in. tapering to 5½ in. in 12 in., the rest of the tower being of uniform 5½ in. bore. The flange at the top of the tower is fitted with three adjusting screws working against the top of the stand, by means of which the tower is levelled.

The spray table with universal adjustment (Pl. 8 *c* and Text-fig. 2).

In order that the correct deposition and distribution shall be maintained, the bottom of the tower must be covered by a plate adjusted so that it is centrally placed and at a given distance from it. Since this gap is too narrow to allow the insects to be placed in position at the bottom of the tower it must be possible to lower the plate, put the insects on it, and raise it again to the correct position quickly and easily. The following arrangement is used: The spray plate (*T*) on which the insects are placed, either in a dish or otherwise, is a metal circle of 6½ in. diam., thus fitting exactly over the bottom of the spray tower. The spray plate carries four adjustable angle-brackets (*AB*) to hold the spray dish, and adjustments are present for centring the dish under the bottom of the tower. From the centre of the spray plate, a pillar (*P*) of $\frac{1}{4}$ in. brass rod runs through a hole bored in a solid metal cylinder ($\frac{5}{8}$ in. diam.)

which is itself fitted in a hollow tube (Tu_1) of the same diameter. The pillar can be adjusted to the requisite height in the solid metal tube then fixed with the Grub screw (g); by this means the gap between the bottom of the tower and the plate can be accurately adjusted. The tube Tu_1 is fitted into a second tube (Tu_2) $\frac{3}{4}$ in. ext. diam. in which it is a sliding fit. The tube Tu_2 fits inside a wide tube

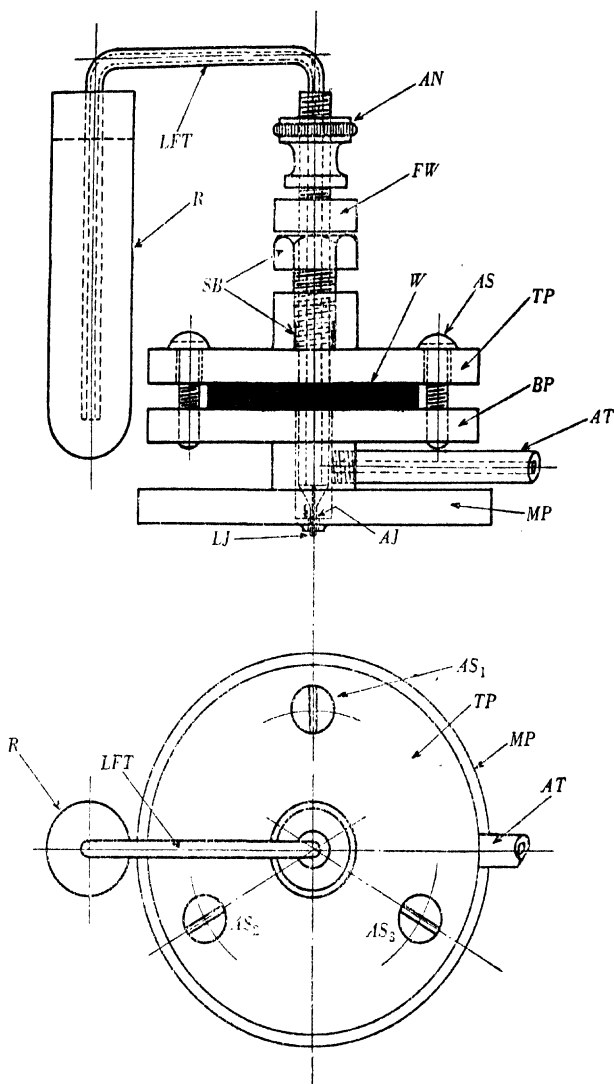


Fig. 1. Cross-section and plan of atomizing nozzle. Scale full size.

AJ, air jet; *AN*, adjusting nut; *AS*₁, *AS*₂, *AS*₃, adjusting screws; *AT*, air tube; *BP*, bottom plate; *FW*, friction washer; *LFT*, liquid feed tube; *LJ*, liquid jet; *MP*, mounting plate; *R*, reservoir; *SB*, stuffing box; *TP*, top plate; *W*, rubber washer.

or cylinder (Tu_3) $2\frac{1}{2}$ in. ext. diam. and 4 in. high and is held in position by six centring screws (S_1 – S_6) arranged to project radially into Tu_3 , three screws at the top and three screws at the bottom. The tube Tu_2 is prevented from falling through tube Tu_3 by the flange *FL*. The tube Tu_3 is mounted on a base plate (*BP*) which has a $\frac{1}{2}$ in. hole, to allow the passage of tube Tu_1 and Tu_2 .

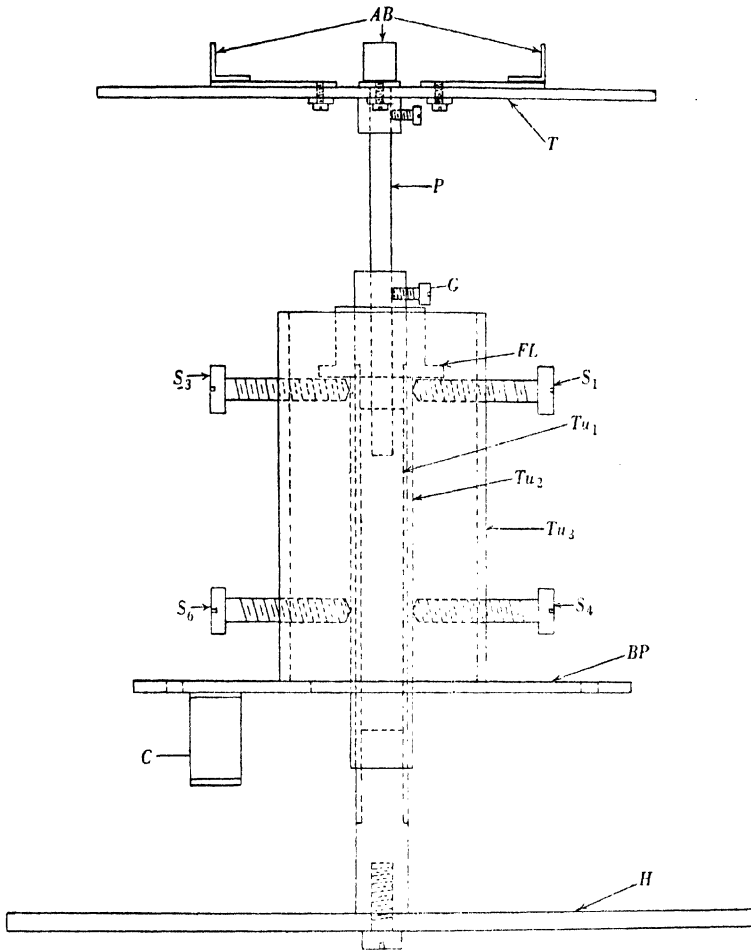


Fig. 2. Spray table with universal adjustment. Longitudinal section. Scale half-size.

AB, angle brackets; *BP*, base plate; *C*, catch; *FL*, flange on *Tu₂*; *G*, grub screw to hold pillar *P*; *H*, handle; *P*, pillar; *S₁-S₆*, centring and adjusting screws; *T*, spray table; *Tu₁*, inner sliding tube; *Tu₂*, outer fixed tube; *Tu₃*, cylinder to hold centring and adjusting screws.

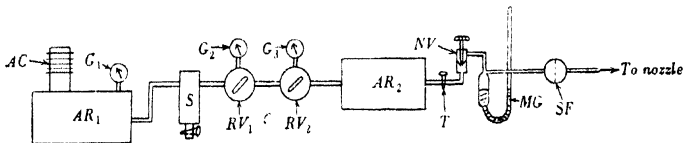


Fig. 3. Diagram of compressed air supply to nozzle, showing system of filters, pressure regulators and stabilizers.

AC, air compressor; *AR₁*, air receiver of air compressor; *AR₂*, small air receiver to equalize flow; *G₁*, first pressure gauge; *G₂*, second pressure gauge; *G₃*, third pressure gauge; *MG*, mercury pressure gauge; *NV*, needle valve regulator; *RV₁*, first reducing valve; *RV₂*, second reducing valve; *S*, oil separator and filter; *SF*, sintered glass filter; *T*, tap.

The base plate is fixed to struts on the stand holding the tower so that the spray table is as nearly as possible directly underneath the tower. The final centring of the spray plate beneath the tower is done by means of the screws S_1 - S_6 . In spraying practice, the tube Tu_1 is allowed to slide down to its full extent in tube Tu_2 , leaving a wide gap between the bottom of the tower and the plate. The insects are put on the plate and the latter then raised by pushing up the tube Tu_1 by means of the handle H , which, when it is raised to the full extent is engaged by a turning movement in the rectangular catch C . Thus with any given adjustment the plate is locked in a position with the gap always the same between the bottom of the tower and the spray plate. With a gap of $\frac{1}{2}$ in. with the table up, there is a gap of $2\frac{1}{2}$ in. with it down, i.e. there is a 2 in. movement. Any necessary levelling of the spray table can also be done with the screws S_1 - S_6 .

The stand.

The whole apparatus is mounted in a rectangular wooden stand (Pl. 1 c), 41 in. high with sides $15\frac{1}{2}$ in. long. It consists of four wooden uprights of 2 in. cross-section. At both top and bottom there are four cross-pieces of the same material; on the top of the stand is a platform of five-ply with a hole in the centre through which the tower fits; the adjusting nuts through the top flange of the tower rest on brass pieces on this platform. The struts to take the base plate of the spray table mounting are fitted to the four uprights.

The compressed air supply.

The compressed air is supplied by an air compressor and is led to the nozzle through a system of filters and pressure regulators and stabilizers as indicated in the diagram (Text-fig. 3). Compressed air from the air receiver (AR) of the air compressor is led through the following units: (1) a separator and filter (S) where the main impurities of oil and water are removed, (2) a first valve (RV_1) to reduce the pressure to near that required, (3) a second valve (RV_2) further to reduce fluctuations in pressure, (4) to a small air receiver (AR_2) to equalize the flow, (5) through a tap (T), (6) through a needle valve regulator (NV) which finally stabilizes the pressure, (7) through a mercury pressure gauge (MG), and finally (8) through a sintered glass filter (SF) to remove the last trace of dust and dirt before reaching the nozzle. Pressure gauges G_1 - G_3 are inserted in order to check the pressure. The elaborate arrangements for stabilizing the pressure are necessary when low pressures of the order of 20 cm. of mercury and less are used; also the slightest particle of dust or other matter is liable to stick in one side of the nozzle and interfere with the distribution and replication of the spray deposit.

Setting up the apparatus for use.

The tower is first levelled by means of the three adjusting screws in the top flange so that the plummet of a plumb-line suspended from the centre of the top falls in the centre of the bottom of the tower. The nozzle is then centred in the top of the tower and roughly levelled. The spray plate is centred over the bottom of the tower and levelled, so that the gap between it and the bottom flange is equal all the way round, this gap being then adjusted to the required distance. For final exact adjustment of the nozzle, it is necessary to do distribution tests with cover-slips, but the rough adjustment normally gives a fairly good distribution.

Physical performance of the apparatus

Method of testing.

When methods of testing the physical performance of the apparatus were first considered an attempt was made to adapt photometric methods for this purpose. A considerable amount of experimental work was done in spraying coloured solutions on to glass plates, the density and evenness of the deposit being measured by passing a beam of light through on to a photo-electric cell and measuring the current produced on a galvanometer. Various difficulties, however, were encountered, and it became obvious that much work would be necessary before a satisfactory practical technique could be hoped for. The method was therefore abandoned and the more laborious but more direct method of weighing was adopted. The

following procedures were used for measuring the errors of replication and the errors of distribution of the deposit.

For replication of deposit. The apparatus was set up for use and the pressure adjusted. A glass Petri dish 8.6 cm. diam. with walls 1.5 cm. high and covered with a ground plate lid was weighed and the Petri dish then placed on the spray table. 5 c.c. of the liquid to be tested was placed in the reservoir of the atomizer and sprayed through, the dish wiped, the lid put on and the whole reweighed. The deposit on the dish was thus obtained.

For distribution of deposit. A glass plate of 9 cm. was covered by a circle of paper which was marked out with a circle of 2.3 cm. diam. at the centre and three other similar circles equally distributed round the circumference. They were numbered 1, 2, 3, 4. Four small brass washers, each approximately 1 cm. diam., were placed at the centre of each of the circles.

Four cover-slips each of 2.3 cm. diam. were placed in numbered weighing bottles and weighed, and put on top of the washers in the circles on the glass plate. The glass plate was then placed on the spray platform and 5 c.c. of liquid sprayed on to it; it was then removed to a table, the cover-slips carefully lifted off with curved forceps, placed in their appropriate weighing bottles and reweighed. The plate was always put on the spray table so that no. 3 cover-slip on the circumference was toward the front of the apparatus. The washers served the purpose of raising the cover-slips so that none of the deposit would be drawn off the surface by the paper and they also facilitated lifting with the forceps. After each spraying the bottles and cover-slips were dried and cleaned carefully and the variation in the series shown in Table 2 between one set of weighings and the next may be in part due to lack of attention to this detail. The technique is laborious and subject to error, especially after some hours work.

Results of tests for errors of replication and distribution of deposit.

Distilled water was selected as the liquid since water besides forming the basis of all aqueous sprays is probably the most difficult liquid to atomize efficiently and distribute evenly.

Tables 1 and 2 give the figures for replication and distribution obtained in the apparatus finally decided on before it was set up for practical use. These figures were obtained working at very low pressures at which the replication might be at its best and the distribution at its worst.

TABLE 1. *Preliminary tests for replication of deposit*

Liquid used = distilled water. Amount used = 5 c.c.
 Pressure = 12.02 cm. Hg. Room temperature = 64° F.
 Room rel. humidity = 70 %. Gap between spray table and bottom of tower = 0.75 in.
 Glass dish = 8.6 cm. diam. with walls 1.5 cm. high.

Weighing no.	Total deposit mg.	Deposit mg./sq. cm.
1	467.0	8.036
2	462.0	7.950
3	459.5	7.907
4	468.0	8.054
5	456.0	7.847
6	458.5	7.890
7	438.5	7.546
8	462.0	7.950
9	448.0	7.710
10	462.5	7.959

Mean total deposit = 458.2 mg.

s.e. \pm 8.944.

Area of dish = 58.11 sq. cm.

Mean deposit per sq. cm. = 7.885 mg.

Proportional s.e. = 1.952 %.

TABLE 2. *Distribution preliminary trials; conditions as in Table 1*

Test no.	Position 1 (centre) mg.	Position 2 mg.	Position 3 mg.	Position 4 mg.	Means mg.
1	30.2	32.1	28.7	28.7	29.92
2	26.7	28.5	27.7	26.9	27.45
3	29.5	29.5	27.7	28.8	28.88
4	30.8	32.5	28.7	27.4	29.85
5	32.1	31.0	29.7	29.2	30.50
Means	29.86	30.72	28.5	28.2	29.32

S.E. of a single deposit $\pm 0.98 = 3.34\%$ of the mean.S.E. of mean of five deposits at one position = ± 0.44 .S.E. of mean of four deposits at one trial = ± 0.49 .

From the figures the replication of deposit appeared satisfactory. The distribution was fair, but the deposit on the cover-slips varied rather widely from one set of weighings to the next, partly because the determinations were carried out over two days, during which the atmospheric conditions varied, and partly to the difficulties of technique. The gap between the spray table and the bottom of the tower was $\frac{3}{4}$ in. for these determinations. The tower was now set up ready for practical use and a series of weighings were made to find out the relationship between the air pressure and deposit and how the replication of deposit varied at different air pressures. (Tables 3-8.)

TABLES 3-8. *Tests to show the variation in replication of deposit at different air pressures and the relationship between air pressure and deposit*

Liquid used = distilled water.

Amount used = 5 c.c.

Room temperature = $74-76^{\circ}$ F.

Room rel. humidity = 45-52 %.

Glass dish 8.6 cm. diam. with walls 1.5 cm. high.

Gap between spray table and bottom of tower = 0.75 in.

TABLE 3. *Pressure 11.31 cm. Hg*

Weighing no.	Total deposit mg.	Time to spray through sec.	Deposit mg./sq. cm.
1	399.0	22	6.866
2	398.5	22	6.858
3	413.0	21.5	7.107
4	407.5	22	7.013
5	415.0	21.5	7.142

Mean total deposit = 0.4066 g.

S.E. ± 0.007677 .

Mean deposit per sq. cm. = 0.00699 g.

Proportional standard error = $\pm 1.888\%$.TABLE 4. *Pressure 19.47 cm. Hg*

Weighing no.	Total deposit mg.	Time to spray through sec.	Deposit mg./sq. cm.
1	333.5	17	5.739
2	329.0	17	5.662
3	337.5	16.5	5.808
4	335.0	17.5	5.765
5	333.5	17	5.739

Mean total deposit = 0.3337 g.

S.E. ± 0.003094 .

Mean deposit per sq. cm. = 0.005742 g.

Proportional standard error = $\pm 0.9272\%$.

TABLE 5. *Pressure 27.20 cm. Hg*

Weighing no.	Total deposit mg.	Time to spray through sec.	Deposit mg./sq. cm.
1	292.5	14	5.034
2	310.5	14	5.343
3	304.5	13.5	5.240
4	307.0	13.5	5.283
5	301.5	14	5.188

Mean total deposit = 0.3032 g.

S.E. ± 0.006834 g.

Mean deposit per sq. cm. = 0.005218 g.

Proportional standard error = ± 2.254 %.TABLE 6. *Pressure 37.78 cm. Hg*

Weighing no.	Total deposit mg.	Time to spray through sec.	Deposit mg./sq. cm.
1	276.5	11.5	4.758
2	290.5	11.5	4.999
3	281.5	11	4.844
4	289.7	11	4.985
5	271.0	11.5	4.664

Mean total deposit = 0.2818 g.

S.E. ± 0.008410 g.

Mean deposit per sq. cm. = 0.004850 g.

Proportional standard error = ± 2.984 %.TABLE 7. *Pressure 60.20 cm. Hg*

Weighing no.	Total deposit mg.	Time to spray through sec.	Deposit mg./sq. cm.
1	213.5	9.5	3.674
2	215.5	9.5	3.708
3	227.0	9	3.906
4	226.0	8.5	3.889
5	224.0	9	3.855

Mean total deposit = 0.2212 g.

S.E. ± 0.005863 g.

Mean deposit per sq. cm. = 0.003806 g.

Proportional standard error = ± 2.651 %.TABLE 8. *Relationship between air pressure and deposit*

Air pressure cm. Hg	Total deposit average mg.	Deposit mg./sq. cm.
11.31	406.6	6.997
19.47	333.7	5.743
27.20	303.2	5.218
37.78	281.84	4.850
60.20	221.2	3.807

Tables 3-8 show that a wide range of deposits can be obtained by variation of the air pressure and the replication of deposit was approximately the same for all the pressures tested. The pressure of approximately 20 cm. gave exceptionally small (replication) errors, but subsequent determinations showed that this was a chance result and that the normal proportional standard error is from 2 to 3 % throughout. The figures also show that the deposit is especially sensitive to changes in air pressure at low air pressures; hence the necessity, when working at low air pressures, of careful stabilization of pressure.

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An air pressure of approximately 20 cm. was then decided on for a normal working pressure because it gave a sufficiently heavy deposit with the 5 c.c. of liquid normally used, and slight differences of air pressure were not so likely to be important as when a low pressure was used. A distribution test was done at this pressure and the figures are given in Table 9. In order to obtain these figures the gap was adjusted to $\frac{1}{2}$ in. and a further test was made to ascertain whether this had interfered with the replication, the results being given in Table 10. The figures for distribution when examined statistically show that under these conditions there is no significant variation of distribution over the area tested, which was a circle of 9 cm., i.e. the area used in practice.

TABLE 9. *Distribution (apparatus set up for use)*

Room temperature = 65° F. Room rel. humidity = 68 %.
5 c.c. distilled water. Pressure = 18 cm. Hg.
Gap between spray table and bottom of tower = 0.5 in.

Mg. per coverslip 2.3 cm. diam.

Time to spray	Position 1 centre	Position 2	Position 3	Position 4	Mean
sec.	mg.	mg.	mg.	mg.	mg.
17	26.0	24.4	24.8	26.2	25.35
16.5	23.2	24.5	24.9	24.0	24.15
16.5	23.9	24.7	25.0	24.1	24.42
16.5	23.6	25.0	25.1	25.2	24.72
16	24.0	25.8	26.0	24.2	25.00
Means	24.14	24.88	25.16	24.74	24.73

S.E. of a single deposit = 0.76 = 3.07 % of the mean.

S.E. of the mean of five deposits at one position = 0.34.

S.E. of the mean of four deposits at one trial = 0.38.

TABLE 10. *Replication under similar conditions*

Time to spray	Mg./dish	
sec.	8.6 cm. diam.	Mg./sq. cm.
16.5	354.5	6.100
16.5	370.5	6.376
16	351.0	6.040
16	348.5	5.997
16.5	355.5	6.118

Mean total deposit = 0.3560 g.

Mean deposit per sq. cm. = 0.006126 g.

S.E. ± 0.008573 .

Proportional standard error = ± 2.408 .

It appears therefore that under optimum conditions the apparatus is capable of repeating a given deposit with a proportional standard error of 2-3 % and giving at the same time a distribution where no significant difference in evenness is shown by the method of demonstration used.

Since much work has been and is being done on insecticides carried in a petroleum oil medium, a few tests were done on the replication of deposit, using first a light oil of the fly spray type and secondly a heavy white oil of the type used as an industrial insecticide in warehouses. No distribution tests were done with these oils, since, as water is far more difficult to distribute evenly, the distribution of the oils might be expected to be as good or better than that obtained with distilled water.

TABLE 11. *Replication using a light oil*

Room temperature = 64° F. Room rel. humidity = 70 %.
 Pressure = 17.97 cm. Hg. Amount in reservoir = 5 c.c.
 Glass dish = 8.6 cm. diam. with walls 1.5 cm. high.
 Gap between spray table and bottom of tower = 0.5 in.

Weighing no.	Time to spray sec.	Total deposit mg.	Deposit mg./sq. cm.
1	20.5	251.0	4.319
2	21.0	263.0	4.526
3	20.5	259.5	4.466
4	21.0	252.5	4.345
5	21.0	253.0	4.354
6	21.0	252.5	4.345
7	20.75	255.5	4.397
8	21.0	245.5	4.225
9	21.5	249.5	4.294
10	21.5	252.5	4.345

Mean total deposit = 0.25345 g.

S.E. \pm 0.004906.

Mean total deposit per sq. cm. = 0.004362 g.

Proportional S.E. = \pm 1.936.

The light oil used has the following specification: sp.gr. 0.779; flashpoint closed, 158° F.; visc. redw. 1 at 60° F., 32 sec.; initial boiling-point, 200° C.; final boiling-point, 270° C. Table 11 shows the results of replication tests under similar conditions to those under which water sprays were tested and the errors are very similar.

TABLE 12. *Replication using a white oil*

Room temperature = 71° F. Room rel. humidity = 52 %.
 Pressure = 33.64 cm. Hg. Amount in reservoir = 5 c.c.
 Glass dish = 8.6 cm. diam. with walls 1.5 cm. high.
 Gap between spray table and bottom of tower = 0.5 in.

Weighing no.	Time to spray sec.	Total deposit mg.	Deposit mg./sq. cm.
1	77.5	115.5	1.988
2	77.5	115.0	1.979
3	78.0	118.5	2.039
4	77.5	118.5	2.039
5	77.5	119.0	2.048

Mean total deposit = 0.1173 g.

S.E. \pm 0.001890.

Mean total deposit per sq. cm. = 0.002018 g.

Proportional S.E. = \pm 1.611 %.

The heavy white oil used has the following specification: sp.gr. 0.862; flashpoint closed, 320° F.; flashpoint open, 335° F.; visc. redw. 1 at 70° F., 118 sec.; pour test = -30° F. Unsulphonatable residue, 99.2 %. This oil which is nearly odourless and tasteless is used in industrial insecticides in warehouses for the protection of stored foodstuffs; it is much more viscous than the light oil and atomizes very slowly at the air pressure used for water sprays, so the pressure was raised and a short series of tests done. Results are given in Table 12. In this short series the errors were smaller than with either water or a light oil, but it is probable that with a longer series the errors would increase and be of the same order as that of the series obtained with the other two. The nozzle appeared to atomize both the oils efficiently.

Adjustment of the amount of deposit.

The figures show the possibility of obtaining any deposit that is likely to be required. Two methods are available for adjusting the amount of material deposited on the spray plate; viz. by variation of the amount of liquid in the reservoir and by variation of the air pressure. Major alterations are best effected by the first method and the final adjustment made by the second. By using the needle valve air-stabilizer, the air pressure can easily be adjusted with considerable accuracy and the deposit regulated to within a few milligrams of any desired amount.

There is no doubt that the total weight of deposit is subject to variation from day to day or even between morning and afternoon, even while the air pressure and all the other adjustments are kept as standard as possible. This variation may be due to changes in atmospheric conditions or to slight changes in the adjustments, the most delicate and sensitive of which is the nozzle itself. The deposit should therefore always be checked before the apparatus is used and rechecked if there is any marked change in atmospheric conditions such as temperature and humidity. The checking only takes a few minutes and enables the deposit to be adjusted with a considerable degree of accuracy.

Test insects and rearing methods

If it is desired to control a particular pest by means of insecticides, the only satisfactory method is to make laboratory and field trials on the species causing the damage, but in order to make a general assessment of the value of any substance as an insecticide, it may be sufficient in the first instance to test it on one or two species of insects. The chief desirable characters apart from any questions of susceptibility of a test subject are: (1) That it can be reared continuously throughout the year without any seasonal rhythm. (2) That the life cycle should be short. (3) That as many instars as possible should be suitable for test subjects. (4) That the stage tested should be able to do without food during the period of spraying and observation. Where plant feeding insects are concerned, to these must be added that the plant may be easily reared throughout the year in a condition to support the insect at the required instar.

Although specificity of effect renders difficult the task of recognizing and comparing an insecticide with others, it may not be so hopeless as it might appear. Some species of insects appear, in general, to be highly susceptible and others highly resistant, and although the order of susceptibility may change with different insecticides, the changes do not appear to be so great as to prevent a general classification of insects into groups according to their susceptibility. Some work on this point has been carried out in this laboratory and will be published later. So far as can be ascertained at present, the species of aphides, used for test purposes, have proved susceptible to all substances of any insecticidal value, so that these insects may be regarded, with comparative safety, as being generally susceptible. It is therefore probable that any substance that will not kill these aphides will not be a useful insecticide for immature and adult insects though it is possible that ovicides may not be detected by these methods. For the recognition and preliminary study of an insecticide substance, a suitable species of aphid should be readily and continuously available.

The species chosen for this purpose was the black chrysanthemum aphid, *Macrosiphoniella sanborni* Gill. It cannot be definitely stated that this is the best species for the purpose, but all the information available suggested it as the most likely to give results. It has been found

that this insect may be reared throughout the year, that its resistance is sufficiently high without being too high, and that it is very easily handled. So far, not enough work has been done on the rearing of this species to enable detailed instructions to be given, but the three main points to be observed are: (1) to maintain a stock of healthy chrysanthemum plants throughout the year, (2) to select one or more suitable varieties of plants. Some varieties are much better than others for the purpose of rearing the insects, but so far it is not possible to list the best varieties. In general, they appear to be those with the most sappy growth, the greenhouse varieties being much better than the outdoor varieties. (3) Periodically, healthy individual insects should be selected and isolated from the stock, which is then destroyed and fresh cultures made up from the selected individuals. The times at which this is best done are not fixed, but can easily be judged by the appearance in the stocks of large numbers of undersized, unhealthy looking individuals, for if the stock is then allowed to go on without interference, it degenerates until all the individuals are undersized and usually a very high percentage of undersized winged forms are produced. If, however, the selection procedure is adopted, it appears possible to maintain the stocks in a good condition over a greater part of the year, if not the whole year, and where the stocks have appeared to be in a good condition, very little variation in level of resistance of the population has been found. On some occasions the insects used for one experiment have been raised from a single individual, but no greater homogeneity has been observed than when the stock was started from several individuals, though the inbred stock has not been compared with wild colonies. The adult wingless parthenogenetic viviparous insects are used as test subjects.

During the summer months *Aphis rumicis* L., the black-bean aphid, is a very good species for testing purposes and is a useful addition to *Macrosiphoniella sanborni*, but so far it has only been reared satisfactorily over a short period in the summer, and this period is very uncertain both in duration and in time of the year. Attempts to prolong the period during which adult wingless parthenogenetic viviparous females, the stage used for testing, are present, have proved unsuccessful. The method at present adopted is to grow bean plants in pots which are covered with muslin. When the aphides appear on beans in the open, individuals are taken and placed on the beans in pots and are kept breeding until the normal migration sets in. Judging on the available data the resistance level of *Aphis rumicis* is higher than that of *Macrosiphoniella sanborni*.

Once a substance has been shown to have insecticidal value, it is frequently desirable to test it on other more resistant species of insects, often for the purpose of comparison with other insecticides and to test it on the various instars. For this and other types of insecticide work, it is desirable to have a number of other species of insects available.

Two other species of plant-feeding insects are being reared in this laboratory which show considerable promise as test subjects of greater resistance than the aphides; these are *Plutella maculipennis* Curtis, the diamond back moth, and *Dysdercus intermedius* Dest., a species of cotton stainer. Both these species have been reared continuously without any serious difficulty over two years, and it appears possible under suitable conditions to rear a generation in about six weeks. Very little insecticidal work has yet been done with these two insects. The adult cotton stainer and the fully grown larva of *Plutella maculipennis* both appear to be very resistant to derris insecticides although these are frequently recommended against the latter species. Only preliminary trials have been made on the other instars, but the eggs of *P. maculipennis* appear to be promising test subjects. Until considerably more

insecticidal work has been done to ascertain the full value of these insects as test subjects and more work has been done on their biology, it does not appear worth while to give details of rearing technique.

Owing to the ease with which some of the insects which attack stored goods can be reared in large numbers, attention has so far been concentrated on them as the source of supply of species of higher resistance level than aphides. Such insects can have the following advantages: (1) a large number of generations is produced in the year without any seasonal rhythm; (2) the difficulty of rearing plants is dispensed with, thus making a greater degree of standardization of the conditions possible; (3) a large number of insects can be produced in a small space with the minimum of labour, so that even with space and labour severely restricted, several species with different resistance levels can be reared. Preliminary tests were carried out on twenty species of stored product insects, and it was decided that the most suitable for general work was *Oryzaephilus surinamensis* L., the saw-toothed grain beetle. Several others are thought to be very useful and sixteen species are kept going, some of which have been used from time to time, though they have not yet been thoroughly investigated.

O. surinamensis, the saw-toothed grain beetle, is reared in a room kept at 27° C. and 70% relative humidity, on a diet of rolled oats and sultanas. The food is first fumigated with carbon disulphide to get rid of predacious mites and is then aired in a fume cupboard for at least 24 hr. Glass jars 8 in. high and 4 in. diam. are used for each culture, four cultures usually being started at one time. Food is placed in the jar to a depth of about 4 in. and then 100 adults are placed in the jar which is covered by a muslin top and put in the constant temperature room. Under these conditions the length of the life cycle from the placing of the adults in the jar to fresh adults emerging is approximately one month. When kept on food at 27° C. and 70% relative humidity, the adult beetle normally lives at least six months. Twenty-one out of twenty-five insects, which were isolated on emerging and placed on sultanas in tubes, were still alive at the end of that period. The adults are, however, very susceptible to starvation and die in 3-6 days when kept without food.

The adults of *Oryzaephilus surinamensis* have proved very useful test insects; they are susceptible to moderate concentrations of derris products, pyrethrum, nicotine, and lauryl thiocyanate, and have proved resistant to any of the carrying media so far tried except where a heavy deposit of oil was used; they were resistant to a light deposit. Other adult insects which seem to be of the same order of resistance as *O. surinamensis* L., are *O. mercator* Fauv., *Ahasverus advena* Waltl., *Lasioderma serricorne* F. and *Laemophloeus turcicus* Grouv. Crauford-Benson (1938 b) preferred *Ahasverus advena* as the most suitable test insect from a range of species which was similar to that tried here. He included *Oryzaephilus surinamensis* and *O. mercator*, as being on an equal footing with *Ahasverus advena*, but did most of his work with *Ahasverus*.

There does not appear to be any great difference in the resistance level between the three species, but *A. advena* feeds on moulds, and it was thought that it would be very much more difficult to produce standard conditions in mouldy cultures than in clean ones and for this reason the *Oryzaephilus* spp. were preferred. So far as could be ascertained, there is no reason for preferring *O. surinamensis* to *O. mercator*, but the former was available in considerable numbers at the start of the tests and its use was therefore continued. The relative resistance levels of the three species of insect most used by us, i.e. *Macrosiphoniella sanborni*,

Aphis rumicis and *Oryzaephilus surinamensis* is shown in Tables 19-21 and Text-fig. 5, graphs 6-8. These figures were obtained using pure rotenone as an insecticide.

It is recognized that it is desirable to have available a test insect from all the main natural orders and to be able to do tests on every instar in all these orders; furthermore, that there should be representatives of plant-feeding insects, insects affecting public or animal health and those of industrial importance. This could be done, but would involve much labour and expenditure, which would scarcely be justified at the present stage of our knowledge of the biological action of insecticides. Insects being bred at the moment include representatives of the Rhynchota, Lepidoptera and Coleoptera, and it appears probable that a number of species in each of these three orders will be found which can be reared easily and which can provide test subjects in every instar.

Factors causing variation in dosage-mortality data

It seems to be generally admitted that it is difficult, if not impossible, to make a complete statement of a set of conditions in which a given concentration of a drug at a given dosage will kill a given percentage of a given population of organisms, yet some attempt at this statement has to be made before a reliable technique for obtaining dosage-mortality data can be elaborated. The sources of variation in the determination must therefore be found out as far as possible and their relative importance assessed. A number of workers have published data on one or more of these sources of variation and it is possible to form a general estimate of some of the factors that appear most important, but there is nowhere the assumption that all the factors causing variation have been recognized. It is generally conceded that the best that can be done at the moment is to standardize the conditions of testing as rigidly as possible in the light of existing knowledge and to rely on statistical methods to decide on the significance of the results. In the absence of any complete statement of the sources of error they may be classified under the three heads: (1) errors due to unequal administration of the dose, (2) errors due to variation in insect resistance, (3) errors due to variations in the general technique such as handling and treatment before and after administration of the dose.

Errors due to unequal administration of the dose.

Before any estimates of variations due to other causes can be made, it is necessary to find a technique whereby an equal dose can be administered to each test insect, but owing to the magnitude of the other errors, it is difficult to assess the effect of variation of dosage alone on insect mortality.

All the testing methods available at present both for sprays and fumigants give dosage-mortality data which show a considerable variation. Perhaps the most accurate method of applying a known dosage of liquid contact insecticide is the micropipette method first used by O'Kane *et al.* (1933) and elaborated by Nelson *et al.* (1934). The biological data given by these latter authors, although very constant when averaged, showed considerable variation within each set of tests with a given insecticide at a given dose. Some data on this point were obtained with the apparatus described in this paper. The results of the first series of experiments are given in Table 13. In these experiments the dishes containing the insects were weighed before and after spraying, so that the weight of the deposit was known accurately. The table indicates that under the conditions of the experiment where the concentration of the poison is below a certain threshold value, variation of the deposit within wide limits has

little or no effect. (The experiment was done in a bare glass dish and the heaviest deposit was only a little short of that at which death due to physical causes was likely to occur.) Where, however, there is a concentration of poison above this threshold value, the percentage mortality increases with increase of deposit. It will be seen, however, that the results are very variable and they indicate that there are factors to be considered which either have not been recognized or the relative importance of which is not appreciated.

TABLE 13. *Effect of weight of deposit on mortality*

Test insect: *Oryzaephilus surinamensis* adults.

Insecticide = W. 212 *Derris malaccensis* resin suspended in water with 5 % ethyl alcohol and 0.25 % sulphonated lorol.

Insects sprayed on a bare Petri dish 9 cm. diam. which was weighed before and after spraying.

(a) 0.001 % resin			(b) 0.002 % resin		
Deposit on dish mg.	% badly affected and dead 1st day	% badly affected and dead 2nd day	Deposit on dish mg.	% badly affected and dead 1st day	% badly affected and dead 2nd day
80.5	3.5	8.9	45.5	38.8	26.0
81.0	13.2	2.6	49.5	40.0	26.7
87.5	16.7	9.6	51.0	19.0	23.8
101.0	9.6	6.0	55.5	58.8	39.6
109.0	24.4	6.8	60.0	69.8	47.2
113.0	9.2	12.2	62.0	64.3	39.0
123.5	17.6	2.0	63.0	44.9	14.3
138.5	14.0	8.2	69.0	35.6	18.2
			73.0	94.0	72.0
			73.5	75.0	60.8
			81.5	95.7	66.0
			94.5	40.0	15.1
			102.0	92.5	69.2

Owing to this condition of variation in mortality at a given deposit over successive sprayings, in order to make even an approximate assessment of the variation of mortality with deposit over a range of deposits, a number of experiments have to be made at each point and a curve plotted. The standard technique for obtaining dosage mortality curves in this laboratory is to have five replicas for each point. Curves have been obtained by Callaway & Musgrave (1940) using the apparatus first mentioned in this paper and shown in Pl. 8 *a*. These authors found that with their technique, if they converted the weight of deposit into logs and converted the mortality into probits, they obtained a straight line relationship between increase of deposit and increase in mortality analogous to that between increase in concentration and increase in mortality.

Some further data on the relationship between deposit and mortality are given in Tables 14-18 and graphs 1-5, Text-fig. 4. In these experiments two different techniques were used; in the first, the insects (adult *Oryzaephilus surinamensis*) were placed in bare glass Petri dishes and sprayed, while in the second, circles of tricholene were put in the Petri dishes to cover the bottom and the insects put on the cloth and sprayed. With the deposit kept constant, the toxicity at different concentrations of poison was determined and a curve drawn, the deposit was then altered and similar data obtained, this procedure was adopted for a number of different deposits using the two techniques outlined above.

Where the bare dishes were used, it was found that the curve for a deposit of 85 mg. showed a reduction in mortality at any given concentration from that given with a deposit

TABLES 14-18. *Effect of variation in deposit on percentage mortality and effect of variation in technique on mortality*

Test insects: adult *Oryzaephilus surinamensis* L.

(a) *Insects placed in bare glass dish 9 cm. diam.*

TABLE 14

Deposit approximately 80 mg. (Av. deposit 78.1 mg. = 1.23 mg./sq. cm.)
Carrying medium = 0.5 % saponin solution in water + 10 % ethyl alcohol.
Insecticide = W. 214 Derris resin (*Derris elliptica* Changi).
Temp. = 61° F. Rel. humidity = 58.6.
Date of trial 22 Feb. 1940.

Conc. of resin mg./l.	Log. conc. mg./l.	No. of insects used	% kill	Probit	
Control	—	58	0.0	0.0	
10	1.0000	60	0.0	0.0	
20	1.3010	63	4.8	3.3354	$n = 4$
40	1.6021	63	1.6	2.8556	$\chi^2 = 1.35$
80	1.9031	63	25.4	4.3380	for $P = 0.05$
120	2.0792	52	53.9	5.0979	$\chi^2 = 9.49$
160	2.2041	60	78.3	5.7824	
240	2.3802	56	96.4	6.7995	

TABLE 15

Deposit approximately 80 mg. (Av. deposit 85.0 mg. = 1.34 mg./sq. cm.)
Carrying medium = 0.5 % saponin solution in water + 10 % ethyl alcohol.
Insecticide = W. 214 Derris resin (*Derris elliptica* Changi).
Temp. = 60° F. Rel. humidity = 58.0 %.
Date of trial 13 Mar. 1940.

Conc. of resin mg./l.	Log. conc. mg./l.	No. of insects used	% kill	Probit	
Control	—	61	4.9	—	
40	1.6021	65	14.5	3.9419	
60	1.7782	58	43.8	4.8440	$n = 5$
80	1.9031	60	59.7	5.2456	$\chi^2 = 11.33$
100	2.0000	67	92.2	6.4187	for $P = 0.05$
120	2.0792	69	86.3	6.0939	$\chi^2 = 11.070$
160	2.2041	57	96.3	6.7866	
240	2.3802	88	100.0	[7.7501]	

TABLE 16

Deposit approximately 150 mg. (Av. deposit 155 mg. = 2.44 mg./sq. cm.)
Carrying medium = 0.5 % saponin solution in water + 10 % ethyl alcohol.
Insecticide = W. 214 Derris resin (*Derris elliptica* Changi).
Temp. = 60.7° F. Rel. humidity = 58.0 %.
Date of trial 13 Mar. 1940.

Conc. of resin mg./l.	Log. conc. mg./l.	No. of insects used	% kill	Probit	
Control	—	70	2.86	—	
20	1.3010	66	23.5	4.2808	
40	1.6021	62	56.8	5.1713	$n = 5$
50	1.6990	60	69.1	5.4987	$\chi^2 = 15.93$
60	1.7782	72	94.3	6.5805	for $P = 0.05$
70	1.8451	66	96.9	6.8663	$\chi^2 = 11.070$
80	1.9031	60	100.0	[7.2551]	
100	2.0000	56	96.3	6.7866	

(b) *Insects placed in a glass dish 9 cm. diam. containing a circle of tricholene*

TABLE 17

Deposit approximately 150 mg. (Av. deposit 150.0 mg. = 2.36 mg./sq. cm.)
 Carrying medium 0.5 % saponin solution in water + 10 % ethyl alcohol.
 Insecticide = W. 214 Derris resin (*Derris elliptica* Changi).
 Temp. = 64° F. Rel. humidity = 60 %.
 Date of trial 3 Apr. 1940.

Conc. of resin mg./l.	Log. conc. mg./l.	No. of insects used	% kill	Probit	
Control	—	113	1.77	—	
90	1.9542	104	45.2	4.8794	
100	2.0000	129	51.1	5.0276	$n = 5$
110	2.0414	120	77.9	5.7688	$\chi^2 = 9.57$
120	2.0792	129	73.2	5.6189	for $P = 0.05$
130	2.1139	107	87.6	6.1552	$\chi^2 = 11.070$
140	2.1461	112	88.2	6.1850	
160	2.2041	98	94.8	6.6258	

TABLE 18

Deposit approximately 300 mg. (Av. deposit 309.5 mg. = 4.86 mg./sq. cm.)
 Carrying medium 0.5 % saponin solution in water + 10 % ethyl alcohol.
 Insecticide = W. 214 Derris resin (*Derris elliptica* Changi).
 Temp. = 64° F. Rel. humidity = 60 %.
 Date of trial 3 Apr. 1940.

Conc. of resin mg./l.	Log. conc. mg./l.	No. of insects used	% kill	Probit	
Control	—	102	0.98	—	
50	1.6990	116	26.83	4.3811	
60	1.7782	103	50.00	5.0000	$n = 5$
65	1.8129	107	50.91	5.0226	$\chi^2 = 9.76$
70	1.8451	118	70.03	5.5244	for $P = 0.05$
80	1.9031	108	90.65	6.3225	$\chi^2 = 11.070$
90	1.9542	100	96.96	6.8808	
110	2.0414	116	100.0	[7.5490]	

of 150 mg. These two curves were obtained using insects from the same culture, sprayed on the same day and with the conditions otherwise the same. A curve obtained for a deposit of 78 mg. showed a reduction in mortality from that in which 85 mg. were used but this curve was obtained with a different culture of insects on a different day under different conditions. This reduction appears too great to be accounted for by the decrease in deposit which is very slight and serves to indicate the variation likely to occur from one week to the next and to emphasize the necessity for care before comparisons are made. With the dishes containing tricholene similar results were obtained, the series with a deposit of 300 mg. showing a marked increase in toxicity over that with a deposit of 150 mg., the conditions otherwise being the same.

These experiments also indicate that if the technique is altered the effect of a given deposit is altered. A deposit of 150 mg. in a bare dish gave a much greater mortality at any given concentration than 150 mg. in a dish containing tricholene. The experiments are not strictly comparable, but taken together with others this point is established with a high degree of certainty. In the instance mentioned the reduction in kill when the dishes contain tricholene can be explained by the absorption of surplus spray by the cloth.

From this evidence it is clear that there can be no satisfactory basis for exploring the effect of liquid insecticides until a technique has been worked out for ensuring that each insect receives a known dose of poison. For this reason most of the work described in this paper has been devoted to devising an apparatus capable of delivering an equal dose of insecticide to each test insect and at the same time to treating, without undue fatigue, a number of insects sufficiently large for statistical analyses.

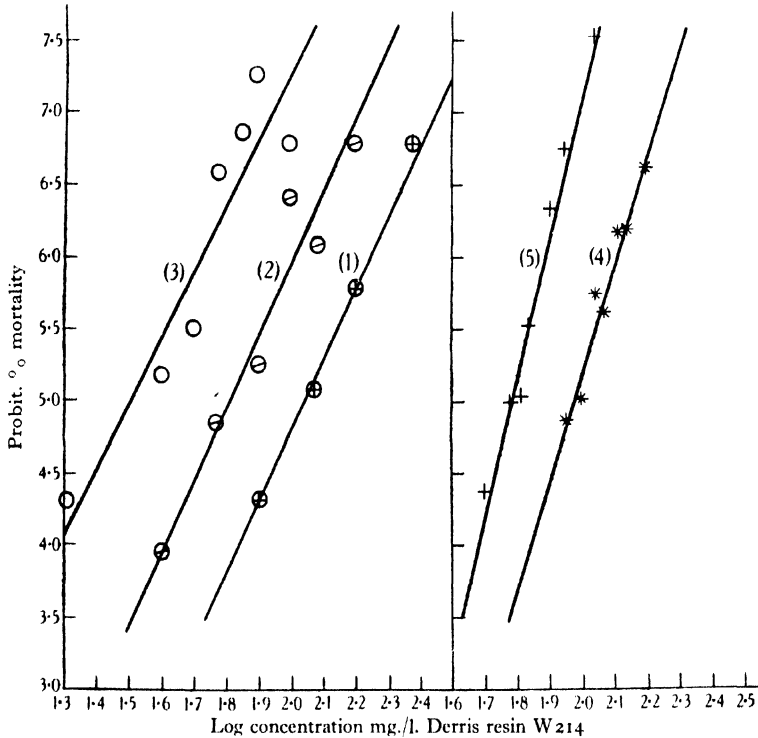


Fig. 4. Graphs nos. 1-5 showing effect of deposit on mortality and effect of technique on mortality. Test insect adult *Oryzaephilus surinamensis*, insecticide Derris resin W214. (1) 78 mg. deposit on bare dish; (2) 85 mg. deposit on bare dish; (3) 155 mg. deposit on bare dish; (4) 150 mg. deposit on dish with tricholene; (5) 309.5 mg. deposit on dish with tricholene. (See Tables 14-18 and text, pp. 160-3, 165.)

Errors due to variation in insect resistance.

Variation in insect resistance may be from one individual to another or from one population to another. Several workers have investigated the sources of individual variation and it has been established with a greater or less degree of certainty that with a given instar of a given species the resistance may vary according to the age and sex of the individuals. The wide individual variation that is found to occur when these two factors are standardized can only be accounted for by what may be termed heredity factors, and there is so far no information adequate to show whether or not this individual variation can be reduced by suitable selection. Nelson *et al.* (1934) found a slight improvement in uniformity through inbreeding from a single pair without any loss of resistance. In this laboratory the aphides used for test purposes have frequently been raised from a single individual, but no com-

parative data have been obtained to show that this results in any greater uniformity in individual resistance than when this technique was not adopted. So far no work has been done to obtain homozygous test insects and to investigate the effect of this on variation of resistance.

It appears certain that the resistance of the population as a whole may change its level to a given poison in a few generations. The reasons for this change of level are not known. Presumably, if it were possible to rear the insects under identical conditions, a stable equilibrium would be reached, but since factors such as population density appear to influence the resistance this is not an easy matter, so that, although by a rigid standardization of rearing technique this variation may be reduced, it must always be recognized as liable to occur. It is possible to approximate to standard conditions of rearing only with insects attacking stored goods. With plant insects the conditions are influenced by the stage of growth of the plant and the conditions necessary for plant health and they are therefore much more difficult to standardize. In this laboratory the insects attacking stored goods are reared in a room kept at a constant temperature and humidity and with the other conditions such as food and population density kept reasonably constant. No attempt has been made to standardize the conditions of rearing of the plant insects; attention has been entirely directed to providing healthy plants in conditions which are to them unnatural and to discover which of these conditions favour the continuous production of healthy insects. Over the period during which our experiments have been made, although some alteration in resistance levels has been observed, there have been no major changes when insects were used which have been carefully reared and which appeared healthy, judged by external appearance.

Errors due to variation in general technique.

In addition to a slow change in the resistance of the population from one generation to the next, differences in resistance may be produced by more immediate conditions of pre-treatment. If insects of a given population are divided into batches and are kept at different temperatures for some time before treatment, there is some evidence to show that those kept at the higher temperatures are less resistant than those kept at the lower. The effect of humidity does not appear to be so noticeable (Gough, 1939). It also appears that the period of removal from their food before treatment has some effect and this should be standardized as far as possible (Gough, 1939; Crauford-Benson, 1938 *a*). No assessment has been made of the effect of handling the insects, but it is quite possible that this may have some effect on their resistance and it is very difficult to ensure that each insect is treated equally in this respect.

There is evidence to show that conditions of after-treatment are also important in affecting the resistance. Here again temperature is the factor chiefly insisted upon, but it would seem that when testing contact sprays in aqueous media, humidity may also play a large part since it affects the rate of evaporation of the carrying medium. Presumably a low humidity causing a rapid rate of evaporation may reduce the effect of the poison, due to its being thrown out of suspensions or solution in the carrying medium and thus preventing penetration into the organism. The humidity of the microclimate around the insects is greatly influenced by the nature of the container in which the test insects are kept after spraying and it is suggested that precautions should be taken to ensure that this microclimate is kept the same in every case.

The effect of variation in the general technique is illustrated by the data set out in Tables 14-18 and the curves shown in Text-fig. 4, graphs 1-5. In the earliest experiments with small insects such as *Oryzaephilus surinamensis* L., *Ahasverus advena* Watl. and *Laemophloeus turcicus* Grouv. a glass Petri dish was used in which the insects were sprayed; they were then kept in the dish until examined. Using a carrying medium of water containing from 5-10% of ethyl alcohol or acetone and either 0.5% saponin or 0.25% sulphonated lorol, it was found that with deposits of much more than 80 mg. per dish of 9 cm. diam., many of the insects tended to stick to the dish. Nearly all the insects so affected were dead and it is not possible to state whether this was partly responsible for their death or not. Some experiments were carried out to compare the effect of spraying *Oryzaephilus surinamensis* in a bare Petri dish with one in which a circle of absorbent material (tricholene) covering the floor of the dish had been placed. In the bare dishes deposits of approximately 80 mg. and approximately 150 mg. and in the dishes containing tricholene of approximately 150 and 300 mg. were tested. The data obtained show the very great effect of an alteration of some factors in the physical environment of the insect during and after spraying when all the other conditions such as dosage, concentration, temperature and humidity are kept the same. The addition of the circle of tricholene not only completely prevented sticking, but compared with a bare dish, it greatly decreased the mortality at a given dose and concentration and altered the slope of the dosage mortality curve, the slope being steeper when dishes containing tricholene were used. The reason for these changes are not clear; reduction in mortality at a given dose is probably due to absorption of the excess spray fluid by the cloth, but it is not easy to understand why the curves are steeper when tricholene is used.

The results using a bare dish with a deposit of 150 mg. gave a high χ^2 value and this heterogeneity was probably due to the extent to which the insects were stuck to the dish. By using a circle of tricholene, this difficulty was prevented, the test was made more sensitive and a very wide range of deposits might be used. This technique was therefore adopted.

The considerable difference in mortality that can be obtained with a given concentration of poison by varying the conditions of spraying such as the weight of deposit and nature of environment, emphasize the futility of translating directly the results of laboratory experiments to recommendations for field use and of attempting to compare the toxicity of insecticides that have not been tested under conditions which are as nearly identical as possible. With any technique using spraying or dipping methods it is difficult to ensure that each insect remains under comparable conditions after treatment. It may remain on a surface wet with the poisonous liquid or crawl on to a part of the container which is dry and it does not appear possible to arrange for complete uniformity of environment after spraying.

The factors outlined are recognized or suspected of causing variation in response to poisoning, but there may also be others of great importance which have not so far been discovered. The most that can be done at present is to standardize the technique as much as possible, and by this means to attempt to control the important variables, both known and unknown.

SPRAYING TECHNIQUE

The technique at present adopted varies slightly with the species of insect. It is not possible to state rigid rules which will apply to all species of insects since some alterations may be necessary with different species and for different instars, but two examples, those of

the insects most frequently used in this work, should make clear the procedure in all its essential points.

(a) *For aphides, e.g. Macrosiphoniella sanborni Gill. and Aphis rumicis L.*

(1) In all tests the adult parthenogenetic viviparous females are used. The insects are removed from the plant and placed in glass tubes a few hours before spraying, ten individuals to a tube. With *Aphis rumicis* the plants are cut the previous day and placed in a large glass jar overnight; by the morning a large number of the individuals required are wandering and are transferred to tubes by means of a camel hair brush. If the insects are removed directly from the plant to the tube while still feeding, there is liable to be risk of injury to the insect by damaging the mouth parts. With *Macrosiphoniella sanborni* it is only necessary to tap the plants for the individuals required for test to fall off. They are thus easily collected in a container from which they are transferred to tubes. By these methods adequate randomization is secured if there are large numbers of insects in good condition, but a further randomization is made by mixing the tubes.

(2) The nozzle of the apparatus is centred and the gap at the bottom checked. About 25 c.c. of the control solution is then sprayed through to get an equilibrium set up in the apparatus and the deposit is checked by weighing. Once the apparatus has been set up and standardized, it is usually only necessary to do from three to five weighings to obtain the required deposit and to check it. A standard Petri dish is used, 8.5 cm. diam. with a ground-glass cover. For most general purposes, 5 c.c. of insecticide are placed in the reservoir and the pressure adjusted to give deposits of the order of 300–350 mg. in the apparatus described, this requiring an air pressure of 14–16 cm. of mercury, using a gap of 0.5 in. between the bottom of the tower and the spray plate.

(3) The insects are transferred from the tubes to Petri dishes containing circles of tri-cholene covering the bottom. They are then placed in the apparatus and sprayed. The spraying is usually replicated five times for each concentration, three replicates being a minimum.

(4) After spraying the dishes are removed, a leaf of the food plant added and the top of the dish is covered with muslin which is held by a rubber band. The dishes containing the sprayed insects are put on trays and removed to a cool damp place, usually a basement room keeping a relatively constant temperature. The insects are inspected the following day and classified as normal, slightly affected, badly affected, moribund, and dead. Fresh food is added and a second inspection is made the day after, if necessary.

Tables 20–21 and Text-fig. 5, graphs 7, 8, show the type of biological data obtained by this technique.

(b) *For insects attacking stored products e.g. Oryzaephilus surinamensis L.*

(1) Cultures are selected which are between 30 and 40 days old; it is estimated that in such cultures any individual adult should not be more than a week old. The adults are removed into tubes, usually twenty insects to each tube on the day before the experiment.

(2) and (3). The procedure is the same as with aphides.

(4) After spraying, the dishes are covered with a muslin cover held in place by a rubber band (no food is added) and removed to the constant temperature and humidity room, each

TABLES 19-21. *Typical curves obtained with the final technique described and showing the resistance levels of Oryzaephilus surinamensis L., Macrosiphoniella sanborni Gill. and Aphis rumicis L. to pure rotenone*

Insecticides used: pure crystallized rotenone.

TABLE 19

Test insect: adult *Oryzaephilus surinamensis* L.

Deposit approximately 300 mg. (Av. deposit 311 mg. = 4.89 mg./sq. cm.)

Carrying medium = 0.5% saponin solution in water + 10% ethyl alcohol.

Temp. = 61° F. Rel. humidity = 52%. Date of trial 10 Apr. 1940.

Conc. of rotenone mg./l.	Log. conc. mg./l.	No. of insects used	% kill	Probit	
Control	—	87	3.5	—	
9.9*	0.996	92	3.1	3.1337	$n=3$
21.0	1.322	93	3.0	3.1192	$\chi^2=5.11$
30.9	1.490	94	11.4	3.7945	for $P=0.05$
40.8	1.611	98	32.3	4.5407	$\chi^2=7.815$
51.9	1.715	93	60.0	5.2533	
61.8	1.791	96	86.1	6.0848	

* Point omitted from calculation of χ^2 and the regression line.

TABLE 20

Test insect: *Aphis rumicis* L. (viviparous parthenogenetic females).

Insects placed in a glass dish 9 cm. diam. containing a circle of tricholene.

Deposit approximately 300 mg. (Av. deposit 327.4 mg. = 5.14 mg./sq. cm.)

Carrying medium = 0.5% saponin solution in water + 10% ethyl alcohol.

Date of trial 9 July 1940.

Conc. of rotenone mg./l.	Log. conc. mg./l.	No. of insects used	% kill	Probit	
Control	—	49	4.08	—	
6	0.7782	48	13.1	3.8783	
8	0.9031	52	29.8	4.4698	
9	0.9542	49	21.3	4.2039	
10	1.0000	50	37.4	4.6787	$n=8$
11	1.0414	52	45.9	4.8970	$\chi^2=5.11$
12	1.0792	54	42.1	4.8007	for $P=0.05$
14	1.1461	50	58.4	5.2121	$\chi^2=16.919$
16	1.2041	48	58.7	5.2108	
18	1.2553	50	70.8	5.5476	
20	1.3010	49	80.9	5.8742	

TABLE 21

Test insect: *Macrosiphoniella sanborni* Gill (viviparous parthenogenetic females).

Insects placed on a tricholene circle, then sprayed and the tricholene circle placed in a 9 cm. Petri dish and the dish covered with damp cellophane.

Deposit approximately 350 mg. (Av. deposit 348.0 mg. = 5.47 mg./sq. cm.)

Temp. = 65° F. Rel. humidity = 75%. Date of trial 16 July 1940.

Conc. of rotenone mg./l.	Log. conc. mg./l.	No. of insects used	% kill	Probit	
Control	—	49	2.04	—	
2	0.3010	47	2.2	2.9859*	
4	0.6021	51	32.0	4.5323	$n=5$
6	0.7782	51	58.0	5.2019	$\chi^2=4.7$
8	0.9031	50	63.3	5.3319	for $P=0.05$
10	1.0000	50	77.6	5.7588	$\chi^2=11.07$
12	1.0792	51	90.0	6.2816	
14	1.1461	49	93.8	6.5382	
16	1.2041	51	100.0	7.0754	

* Point not used in calculating χ^2 and the regression line.

set of five is removed to the room before spraying the next set. They are inspected on the following day and a second inspection is made if necessary the day after.

Tables 17-19 and Text-figs. 4, 5, graphs 4-6, show examples of the type of biological data obtained with this technique.

These two techniques cover the procedure necessary for testing most species of insects, slight modification only being necessary for individual cases. It seems, however, likely that some modifications may produce greater uniformity of results, particularly any that help to standardize the conditions after treatment. Some further experiments have already been made with this end in view.

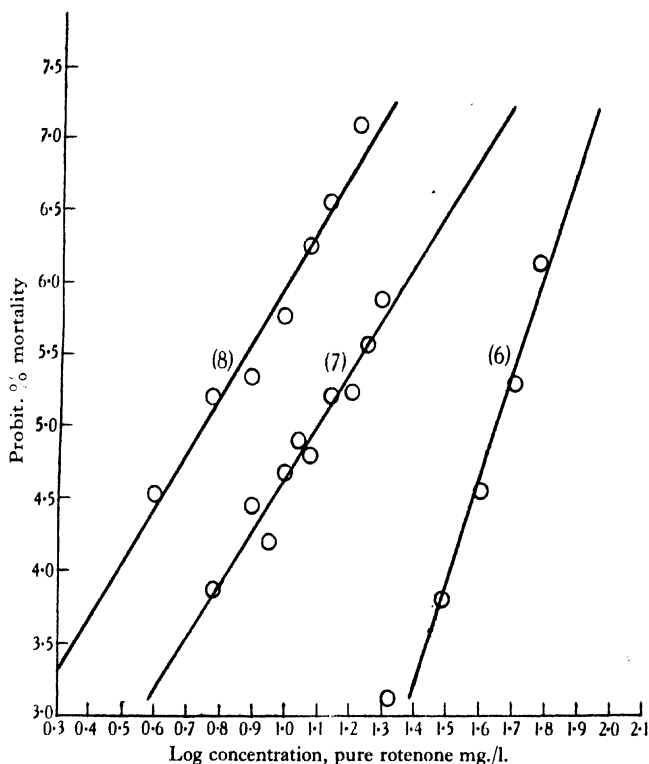
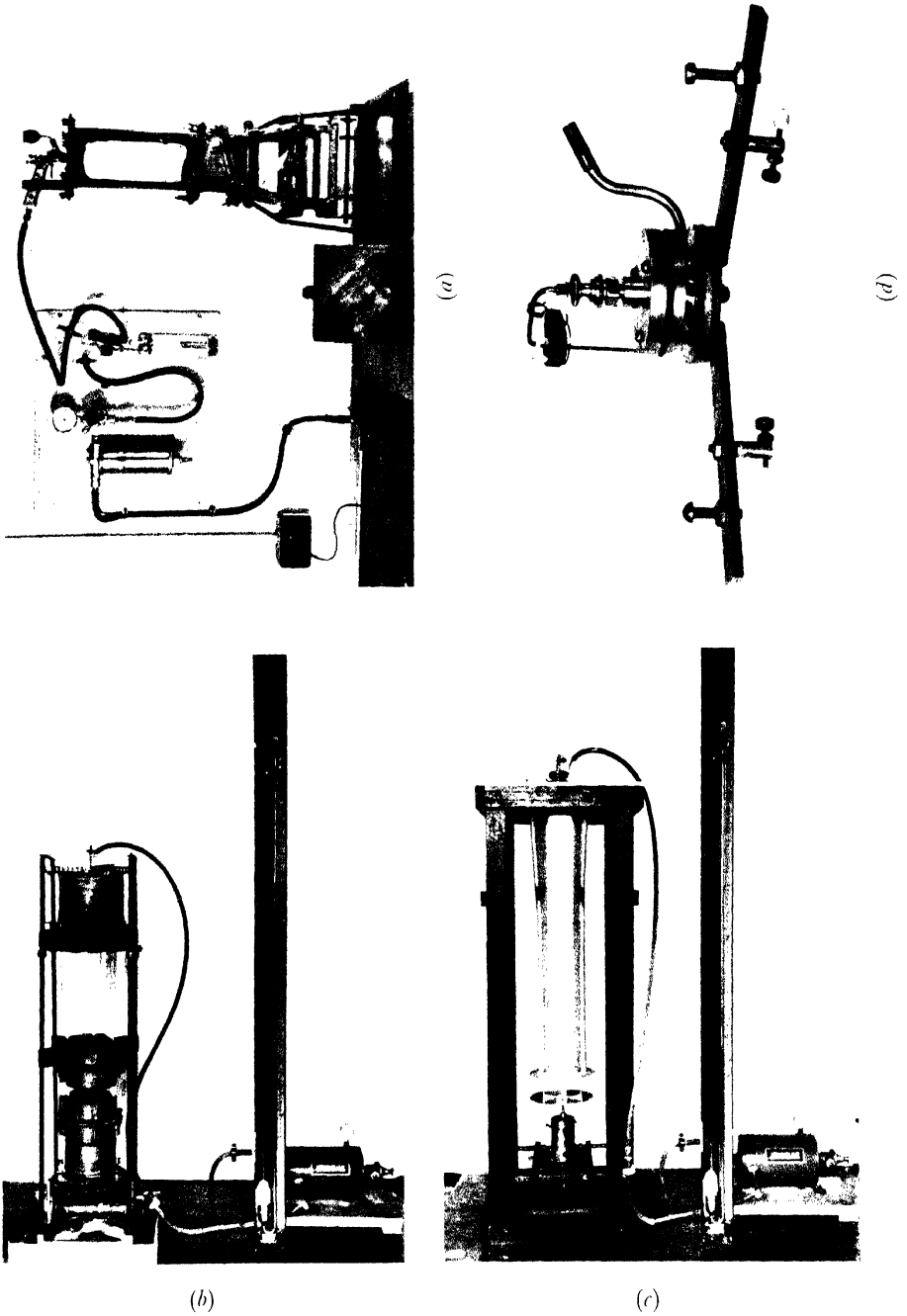


Fig. 5. Graphs nos. 6-8 showing type of data obtained with the technique described, and the resistance levels of three test insects, *Macrosiphoniella sanborni*, *Aphis rumicis* and *Oryzaephilus surinamensis*. Curves obtained using pure rotenone as toxic agent. (6) *Oryzaephilus surinamensis*; (7) *Aphis rumicis*; (8) *Macrosiphoniella sanborni*. (See Tables 19-21.)

SUMMARY

An outline of the general problem of the evaluation of liquid contact insecticides is given. A summary of the laboratory methods of evaluation already described and the reasons for the adoption of the procedure described are outlined. An account is made of the development of the spraying apparatus together with experiments on factors likely to cause variation in the replication and distribution of the deposit. The apparatus finally adopted is described and data are presented on its physical performance. The selection and rearing of test insects is described. An account is made of the factors in the technique which may cause errors in



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THE ACTION OF CONTACT INSECTICIDES (pp. 142-69)

the determination of the dosage-mortality data, with some experiments. The technique at present adopted is described, with examples of the dosage-mortality curves obtained.

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EXPLANATION OF PLATE 8a-d

- (a) Original apparatus with mixing tower and settling chamber. The spray plate which fits at the bottom when in use is shown on the left of the apparatus. The air line with filter, reducing valve, pressure gauge and air-flow meter is shown on the panel.
- (b) Photograph of apparatus used for a considerable amount of experimental work using a glass tower, scent-spray nozzle and spray table without universal adjusting device.
- (c) Photograph of apparatus finally decided upon with metal tower, specially designed nozzle and spray table with universal adjustment.
- (d) Photograph of atomizing nozzle and mounting.

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SOIL CONDITIONS AND THE TAKE-ALL DISEASE OF WHEAT

VI. THE EFFECT OF PLANT NUTRITION UPON DISEASE RESISTANCE

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FIELD observations on the effect of fertilizer treatment upon occurrence of take-all in wheat, found in the literature, are concerned chiefly with the beneficial influence of superphosphate in the control of this disease on Australian soils, some of which are notoriously deficient in phosphate (Griffiths, 1933; Samuel, 1934). Few precision experiments have been reported. Rosen & Elliott (1923) carried out an experiment involving three treatments, viz. farmyard manure at the rate of 10 tons/acre, a 4-8-3 general fertilizer at the rate of 400 lb./acre, and burnt lime at the rate of 1 ton/acre. The percentages of whiteheads on the different $\frac{3}{4}$ acre plots at harvest were as follows: control plot (untreated) 80%, farmyard manure plot 45%, artificial fertilizer plot 7% and lime plot 95%. The average yields per acre were as follows: untreated plot 4 bushels, manure plot 14.3 bushels, fertilizer plot 18.4 bushels, and lime plot a total failure. The action of lime in increasing the disease was elucidated by Garrett (1936), since when some striking examples of its disease-promoting effect have been noted on English farms. The remaining data of Rosen & Elliott indicate that nutrition of the host plant may be a factor of importance in determining the influence of infection upon crop yield. Whilst adequate manuring may not appreciably check the mycelial advance of *Ophiobolus* along the root system to the crown, it may yet minimize the influence of infection upon the plant's growth and yield. Doughty *et al.* (1929), in a survey of a field experiment upon the top dressing of wheat with $1\frac{1}{2}$ cwt. sulphate of ammonia, recorded a mean incidence of 25% of whiteheads in four top-dressed plots, as against a mean of 33% for the four untreated control plots.

The previous papers of this series (Garrett, 1936 *et seq.*) dealt with the effect of soil conditions upon the activity and survival of the causal fungus; the experiment here described is concerned with the effect of soil conditions upon the resistance to disease of the host plant.

EXPERIMENTAL

Wheat plants grown in sand culture under five different manurial treatments were inoculated at a suitable stage with *Ophiobolus* and the development of disease recorded at the end of the experiment, together with its effect upon the yield of grain. The sand-culture technique was adapted from that devised by Gregory & Crowther (1928) for the first of a series of experiments upon the nutrition of the barley plant. Gregory & Crowther grew three barley plants in 30 lb. of sand per pot. In this experiment with *Ophiobolus*, single wheat plants were grown in 7 in. amber glass flower pots holding 10 lb. of washed sand; the amounts of nutrients given per pot were therefore one-third of those given by Gregory & Crowther to each of their pots, but the amounts per plant were the same. Where a pathogenic organism is concerned single plant pot culture has one important advantage over the more usual method of growing three or more plants in a pot; by the latter method, surviving plants in a pot derive a nutritional advantage if one or more of the original complement is killed or seriously retarded in growth by the disease.

Five nutrient treatments were compared, each nutrient series containing eighteen inoculated pots and six uninoculated or control pots. The treatments were as follows:

	N	P ₂ O ₅	K ₂ O
NPK	0.50	0.17	0.33
PK $\frac{1}{3}$ N	0.17	0.17	0.33
NK $\frac{1}{3}$ P	0.50	0.057	0.33
NP $\frac{1}{3}$ K	0.50	0.17	0.11
$\frac{1}{3}$ (NPK)	0.17	0.057	0.11

In the full nutrient or NPK series, each plant received the same amounts of N, P and K as were given by Gregory & Crowther to their barley plants. In three of the remaining series, each of the three nutrients was deficient in turn by two-thirds of the full amount. In the fifth series, all three nutrients were present in only one-third of the full amount. The composition of the full nutrient solution actually employed was similar in respect of all constituents except phosphate to one of those used by Richards & Shih (1940) and was as follows:

	g. per pot
NaNO ₃	1.52
Ca(NO ₃) ₂ .4H ₂ O	2.11
Na ₂ HPO ₄ .12H ₂ O	0.833
CaCl ₂ .6H ₂ O	0.123
MgSO ₄ .7H ₂ O	0.417
K ₂ SO ₄	0.617

Subsequent to planting, an addition was made to each pot of 0.0033 g. MnSO₄ and of 0.037 c.c. of a saturated solution of FeCl₃.6H₂O; each of these was given in 100 c.c. of water.

In setting up the experiment, the complete solution for each manurial series was made up in amount sufficient for the twenty-four pots to be filled, but with omission of the sodium phosphate. The phosphate solution, which had previously been adjusted to pH 6.9, was added to the aliquot of nutrient solution measured out for each individual pot, and the resulting fine suspension shaken and poured onto the sand. After all the pots had been set up, three seeds of Red Marvel spring wheat were sown in each pot on 26 Mar., and the resulting seedlings thinned to one per pot one week later. The pots were randomized on four parallel benches in a large compartment of the glasshouse.

The question of inoculation received particular consideration. In the field, the degree of injury caused by the disease must largely depend upon the stage of plant growth at which the fungus reaches the crown of the plant. The importance of the spatial interval between inoculum and crown of the plant was demonstrated by Fellows & Ficke (1934) for one particular soil type. The time interval between contact of the root with the inoculum and infection of the crown by the fungus depends, however, not only upon the spatial interval between inoculum and crown, but also upon rate of growth of the fungus along the roots (Garrett, 1936). This relationship was well demonstrated by the experiments of Winter (1939), who also derived mathematical formulae expressing the relationship of disease injury to amount and distribution of inoculum in the soil, on the one hand, and to soil conditions and the resulting growth rate of the runner hyphae, on the other (Winter, 1940).

Although plants may be killed in the early stages of growth in the field, the disease is more commonly expressed in the development of whiteheads; production of the disease in this form was therefore made the object of this pot experiment. If the inoculation were to take effect too soon, the plants would be killed before heading; if too late, grain would develop in the ears before the disease had had time to cause serious interference. It was finally decided to bury a 0.25 cm. layer (50 g.) of sand + 3% cornmeal inoculum at a depth of 10 cm. below the seed and 5 cm. above the bottom of the pot; the seed was planted at a depth of 2 cm. Unfortunately, this inoculation was rendered completely ineffective through waterlogging. During the first 3 weeks of the experiment, the moisture content of the sand was maintained at 70% saturation, the drainage hole at the bottom of the glass pot having been plugged with paraffin wax. Insufficient allowance was made, however, for the sinking of the water in the sand, which proceeded to such an extent as to involve the inoculum layer in the saturated or subsaturated zone. For this reason, presumably, no infection occurred in any pot from the original inoculum layer, as was verified at washing out of the roots after harvest. On 1 May, approximately one month after planting, the seedlings were reinoculated by the insertion of two 1 in. lengths of infected wheat straw into the sand on either side of and immediately below the crown. This second inoculation proved to be effective and fortunately timed.

In the earlier stages of growth, but not later, the plants required support from a wire ring. The moisture content of the sand was maintained at 70% saturation for the first 3 weeks, but after that, the paraffin plugs were removed from the drainage holes, and the pots stood in glass saucers and watered from a can. Neither rust nor mildew developed on leaves or stem of any plant, nor did any mould growth subsequently appear upon the whiteheads. A severe infestation of aphides threatened to develop, but was checked by fumigation with nicotine on two consecutive occasions. A test exposure of growing Petri dish colonies of *Ophiobolus* to a nicotine fumigation had previously shown that the growth of the fungus suffered no perceptible check from the fumigant.

The ears emerged during the first week of June; towards the end of the month, whiteheads appeared in series $\frac{1}{3}$ (NPK) and subsequently in other series. No precise count of whiteheads was possible, however, owing to the various gradations between completely empty whiteheads and well-filled heads. The ears of the plants were not harvested until the ear-chaff had completely lost every trace of green and the ears were judged to be almost dead ripe; the phosphate-deficient series, $\text{NK}\frac{1}{3}\text{P}$, were some days later in ripening, and were harvested a few days after the other series. The ears were placed in labelled paper bags; the grain was rubbed out of each ear and weighed one month later.

After harvesting the ears, the root systems of the plants were washed out for examination. At this time, the pH of the sand was tested in three pots of each series; values were just over seven in almost every pot, and the reaction of the sand would therefore have been favourable to the progress of infection in every series. Inspection of the root systems after washing out showed that satisfactory infection of the roots from the straw inoculum had occurred in every inoculated pot of the experiment. After washing out, the plants were split up into their constituent tillers, and classified according to the degree of visible infection of tiller-base and root system. The number of tillers with *Ophiobolus*-blackened stem-bases was recorded for each experimental series; doubtful cases in which blackening was just commencing were given a half mark, and the halves subsequently added up to the nearest whole number. At the same time, the number of tillers with majority of roots severely infected was also recorded for each series. In the remaining tillers the root systems were also extensively infected, but a majority of the roots could be classed as only moderately to lightly infected, with a proportion which were apparently healthy (see Table 1).

TABLE 1. *Degree of visible infection of stem-base and root system of ear-bearing tillers*

	Mean no. of ear-bearing tillers per plant	% tillers with <i>Ophiobolus</i> - blackened stem-bases	% tillers with majority of roots severely infected
NPK	5.5	20	66
$\text{PK}\frac{1}{3}\text{N}$	4.3	21	35
$\text{NK}\frac{1}{3}\text{P}$	4.9	41	68
$\text{NP}\frac{1}{3}\text{K}$	4.8	39	79
$\frac{1}{3}$ (NPK)	4.3	52	65

In accordance with expectation, deficiency of nitrogen reduced tillering to a greater extent than deficiency of phosphate or of potash. The lowest percentage figures for *Ophiobolus*-blackened stem-bases are those of 20 and 21 for series NPK and $\text{PK}\frac{1}{3}\text{N}$, respectively. Series $\text{NK}\frac{1}{3}\text{P}$ and $\text{NP}\frac{1}{3}\text{K}$ are approximately equal with 41 and 39%, respectively, whilst series $\frac{1}{3}$ (NPK) is highest with over 50% of blackened stem-bases. Infection figures for the root systems agree only in a general way with those for the stem-bases. Thus the percentage of tillers with majority of roots severely infected was of the same order (65–68%) for three series, viz. NPK, $\text{NK}\frac{1}{3}\text{P}$ and $\frac{1}{3}$ (NPK). It was much lower than this for series $\text{PK}\frac{1}{3}\text{N}$ (35%) and somewhat higher for series $\text{NP}\frac{1}{3}\text{K}$ (79%). Thus, whilst a deficiency of P or K, or of all three nutrients together, tends to increase the degree of visible infection, a deficiency of N appears to reduce it. The healthier appearance of the N-deficient root systems at the time of washing out was particularly striking.

Figures for mean grain weight per plant in each series may next be considered (Table 2).

TABLE 2. *Mean grain weight per plant (g.)*

	Control	Inoculated	% reduction in yield due to infection
NPK	8.31 ± 0.37	8.07 ± 0.55	3
PK $\frac{1}{2}$ N	7.88 ± 0.56	5.97 ± 0.37	24
NK $\frac{1}{2}$ P	5.80 ± 0.37	2.93 ± 0.32	49
NP $\frac{1}{2}$ K	8.22 ± 0.28	6.51 ± 0.45	21
$\frac{1}{3}$ (NPK)	6.83 ± 0.17	3.86 ± 0.40	43

The following conclusions concern the significance of the differences in Table 2.

(1) In the control pots, deficiencies of N and of K, respectively, failed significantly to depress the yield below that of the full nutrient series, NPK. Deficiency of P greatly reduced yield; deficiency of all three nutrients, however, resulted in a significantly smaller depression in yield.

(2) In the inoculated pots, a significant depression in yield occurred in all four deficiency series.

(3) Infection significantly reduced yield in every series except that receiving the full nutrient solution, NPK. Percentage reduction in yield due to the disease is highest in series NK $\frac{1}{2}$ P and next in series $\frac{1}{3}$ (NPK).

Of particular interest is the fact that deficiencies of N and of K, which failed significantly to depress yield in the control series, nevertheless significantly reduced yield in the inoculated series. Also of interest is the fact that deficiency of P alone reduced the yield more severely, in both control and inoculated series, than the deficiency of all three nutrients together. Although with this particular scale of nutrients and nutrient deficiencies, phosphate deficiency produced the most striking reduction in yield, it would be unwise to stress too far the parallel between this experimental result and Australian observations. The chief conclusion to be drawn from this experiment is that any nutrient deficiency, if sufficiently severe, is likely to intensify the loss in yield through disease.

SUMMARY

Red Marvel spring wheat plants were grown singly in sand culture in 7 in. glass flower pots, under conditions of full nutrient supply, and under deficiencies of nitrogen, phosphate and potash and of all three together, respectively. After one month's growth, the plants were inoculated with *Ophiobolus* by the insertion of two pieces of infected wheat straw into the sand on each side of and just below the crown.

Satisfactory root infection occurred in every plant inoculated, but was lightest in the nitrogen-deficient plants, whilst the potash-deficient plants exhibited a rather more intense root infection than those of any other series. Percentage infection of the stem-bases was lowest in the full nutrient and in the nitrogen-deficient plants, and highest in the series deficient in all three nutrients.

In the uninoculated control plants, a significant depression in grain yield was produced only by the deficiency of phosphate; in the inoculated plants, however, deficiency in any one of the three plant nutrients significantly reduced grain yield. Infection significantly reduced yield in every series except that receiving full nutrients; the percentage reduction was greatest in the phosphate-deficient plants.

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SOIL CONDITIONS AND THE TAKE-ALL
DISEASE OF WHEATVII. SURVIVAL OF *OPHIOBOLUS GRAMINIS* ON THE ROOTS
OF DIFFERENT GRASSES

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OCCASIONAL observations on the relative susceptibility of different grass species to infection by *Ophiobolus graminis* are to be found in the majority of papers on the take-all disease; extensive experimental tests have been conducted only by Kirby (1922, 1925), Padwick & Henry (1933), Russell (1934) and Winter (1939). The tests were carried out in glasshouse pots; observations were made by Kirby after 5 months in the first and after 1 year in the second test, by Padwick & Henry after 7½ weeks, by Russell after 14 weeks and again after 7 months, and by Winter after 5½ weeks and again after 5 months. All these authors supplemented their pot tests by observations on wild grasses collected from the field. On tabulating their results, and comparing them with a series of three similar tests made by the writer, fair agreement was observed except for the first trial by Kirby (1922). In this trial, Kirby obtained notably fewer positive results than the other investigators, recording as resistant to infection a number of species which were otherwise unanimously agreed to be susceptible. In his second trial, Kirby (1925) expressed his results solely by the relative abundance of perithecia on the inoculated plants after 1 year. This criterion was certainly consistent with Kirby's (1925) expressed view that 'the ascospores constitute the inoculum for the vast majority of infections', though this view has since been challenged (Garrett, 1939). Moreover, Müller-Kögler (1938) has since reported that numerous perithecia may be formed superficially in the tissues of plants resistant to the further progress of infection; this observation has been confirmed by the writer. With the exception of Kirby, therefore, these different investigators agreed that species of the following genera were susceptible to attack, viz. *Agropyron*, *Bromus*, *Festuca*, *Hordeum* and *Lolium*, and also the species *Dactylis glomerata*; as resistant were classified species of *Agrostis* and *Avena* and also the species *Phleum pratense*. No conclusion could be reached concerning the susceptibility of *Poa* spp., which appeared more variable in this respect than species of other genera.

Although infection by *Ophiobolus* may impair the efficiency of individual plants of susceptible grass species, even leading in extreme cases to death of the individual, it is rarely likely to be the factor limiting the productivity of the pasture as an ecological whole. Such loss of productivity can only occur when the dominant species is highly susceptible to attack by *Ophiobolus*; an example is furnished by the almost pure stands of *Hordeum murinum* seen in South Australia. The agricultural importance of grass hosts is to be sought firstly in the origin of the disease in virgin grasslands, and secondly in the indefinite survival of the fungus in temporary pastures from one cereal crop to the next, as well as

on susceptible grass weeds on fallows and in other crops. Neither in Canada nor in Australia is the appearance of take-all in the wheat crops generally long delayed after the plough has first been put into the virgin land. Thus Sanford (1929), after surveying the Alberta wheat crops in 1928 for root rots, chief amongst which was take-all, reported: 'Where wheat follows breaking, the 0.9 (per cent) loss class was not exceeded, and a few of the fields have no recorded loss. These observations correspond with the data from surveys made in 1927 and earlier. However, there is a marked increase in the severity of the disease on the second crop following breaking. The severity of foot-rot in the third and fourth successive crop of wheat following breaking is often very marked, and the distribution of the disease is usually fairly general.' From a similar survey of the cereal crops made in Saskatchewan, Russell (1934) concluded: '*O. graminis* is present in the virgin sod; it seldom damages the first crop of wheat on new land to any great extent, but the second and subsequent crops of wheat may be severely diseased unless the wheat crops are alternated with summer fallow or crops of highly resistant plants.' From South Australia, Griffiths (1933) remarked that take-all 'frequently appears after the first crop, particularly when the foolish policy of growing several wheat crops in succession is adopted. There is no doubt that much of the present trouble has been caused by the general practice in the past of over-cropping new mallee areas with wheat.' An English survey now in progress is showing that *Ophiobolus* is apparently widely distributed in the old grassland areas of this country.

These aspects of the take-all problem appeared to demand further study, in view of the lack of direct experimental evidence as to the relative efficiency of different species of grasses as propagators of *Ophiobolus*. Whilst agreement between the results of the different inoculation trials discussed above had been fairly good, there were some discrepancies. For instance, Winter found that 38 days after inoculation, plants of *Agrostis spica-venti* seemed outwardly healthy; the roots appeared scarcely infected, and runner hyphae were sparsely distributed, in agreement with other observations on the resistance to infection of *Agrostis* spp. Yet, after 5 months, all inoculated plants were dead, and bore perithecia. Again, in tests by the writer, certain species, such as *Anthoxanthum odoratum*, *Cynosurus cristatus* and *Poa* spp., showed a variable amount of root discoloration, and were difficult to classify definitely either as susceptible or as resistant. Even apparently resistant species such as *Avena elatior* or *Phleum pratense* showed occasional discoloured lesions on the roots. In general, infection of the seminal roots was more severe than that of the crown roots in all species examined. Little reliance could be placed on the presence or absence of runner hyphae on the roots as a criterion of fundamental susceptibility, since Müller-Kögler (1938) has shown that *Ophiobolus* hyphae will grow down the tap-roots of many dicotyledonous seedlings, which afterwards throw off the infection. Turner (1940) has demonstrated that the runner hyphae will grow along both seminal and crown roots of oats (albeit less rapidly than along those of wheat), but that infection proceeds no further after initial penetration of the outer root cortex. In these grass host investigations, runner hyphae were observed by Winter and by the writer on the roots both of *Avena elatior* and of *Phleum pratense*, which otherwise appeared to be resistant.

These difficulties were appreciated by Padwick (1935), who remarked 'the mere fact that a plant is susceptible to attack by wheat foot-rotting pathogenes under the unusual conditions of experimental inoculation, especially in sterilized soil, does not give an adequate indication of the role which it may play in the problem of foot-rot of wheat'. Padwick

therefore employed a direct technique for determining the power of different grass species to act as perpetuators of *Ophiobolus*. He sowed seed of four species of grasses, *Agropyron tenerum*, *A. cristatum*, *A. repens*, and *Bromus inermis*, in pots of black loam soil artificially inoculated with *Ophiobolus*; ten pots were sown with each species of grass, and two additional series of pots were included in the experiment, one left fallow and the other sown with *Neslia paniculata* (ball mustard), a dicotyledon. The plants were allowed to grow in the glasshouse for 1½ months, after which the tops were cut off, and twenty-five wheat seeds sown amongst the roots in each pot. After 3 weeks, the wheat seedlings were removed and the degree of infection recorded. No infection was observed on those seedlings in the fallow unsterilized soil, showing that the fungus had died out during the 1½ months' interval between inoculating the soil and planting the test wheat seedlings; in the fallow-sterilized soil a very light infection of the wheat seedlings was recorded. No infection occurred in either soil series planted with the ball mustard. Appreciable infection of the test wheat seedlings occurred after sowing with all four grasses, the maximum disease rating being one of 16%. In another type of experiment, Padwick demonstrated that *Ophiobolus* not only survived but also spread for distances of up to 12 in. in 7½ weeks, from a trench filled with corn-meal soil inoculum, along the roots of *Agropyron tenerum*, of *A. repens* and of wheat, respectively.

Padwick's technique was therefore adopted and extended for the present investigation; whereas Padwick only made one observation on the susceptibility of each grass species, the experiments here described provided for serial observations, through the planting of test wheat seedlings at regular intervals on the inverted sods of the inoculated grasses. No dicotyledons other than *Trifolium pratense* were included in these experiments, since there is no field evidence to suggest that species of plants outside the Gramineae can carry *Ophiobolus*. This conclusion has been confirmed by the studies of Müller-Kögler (1938), who investigated the reaction of seventy-three dicotyledons to inoculation with *Ophiobolus* under soil conditions extremely conducive to infection, and found none to be infected to any appreciable extent. He divided the species into four groups: (1) showing no signs of infection; (2) showing growth of runner hyphae along the exterior of the roots, but a minimum of internal infection; (3) showing rapid infection of the primary root cortex, which was sloughed off by formation of a pericyclic epidermis, with termination of the infection; (4) showing rapid infection of the primary root cortex but no further progress of infection, presumably owing to protoplasmic incompatibility between host and parasite.

EXPERIMENTAL

The general plan of these experiments was as follows. The seed of different species of grasses was planted in wooden boxes over the minimal amount of *Ophiobolus* inoculum necessary to secure uniform infection of susceptible species; after a 2-months period of growth, the grass tops were cut off and the sods inverted in the boxes, to simulate ploughing up. The boxes were stacked in a compartment of the glasshouse maintained as far as possible at a constant temperature; at intervals, test wheat seedlings were planted in the inverted sods of the different grasses, and examined for root infection after 3 weeks' growth. In this way, the degree of survival of *Ophiobolus* on the root system of the different species of grasses could be determined.

Exp. I. The following species were employed:

<i>Agrostis alba</i> var. <i>stolonifera</i>	<i>Festuca pratensis</i>
<i>A. tenuis</i>	<i>F. rubra</i>
<i>Alopecurus pratensis</i>	<i>F. rubra</i> var. <i>fallax</i>
<i>Anthoxanthum odoratum</i>	<i>Lolium italicum</i>
<i>Avena elatior</i>	<i>L. perenne</i>
<i>Cynosurus cristatus</i>	<i>Phleum pratense</i>
<i>Dactylis glomerata</i>	<i>Poa pratensis</i>
<i>Festuca ovina</i>	<i>P. trivialis</i>

The experiment was carried out in wooden seed boxes, $35 \times 22 \times 8$ cm., filled with a mixture of three parts sand to one part of Harpenden allotment soil (by volume). The soil was diluted with sand so as to provide optimum conditions for *Ophiobolus* to infect and spread along the roots of the grasses (Garrett, 1936). Seven boxes were planted with each grass species, and fourteen boxes were left fallow, to serve as a check on the occurrence of the fungus in the unsterilized allotment soil. Inoculation was effected by spreading 150 g. of a sand + 3% cornmeal culture of *Ophiobolus* (isolate obtained from wheat at Wareham, Dorset, in 1938) as a layer approximately 1.25 mm. deep on the surface of the soil in each flat before sowing the grass seed, which was then covered to a depth of approximately 5 mm. with the same soil mixture. The boxes were planted on 24 July 1939, and placed outside in groups of twenty inside wooden frames covered with a wide-mesh white window netting, to keep out sparrows. A standard dose of plant nutrient solution was given to every box at 4, 5 and 6 weeks after planting; the total addition of nitrogen corresponded approximately to a field dressing of 1 cwt. of nitrate of soda/acre.

At the end of 2 months the majority of grasses had made satisfactory growth. Establishment has been good in all boxes, though the two *Lolium* spp. had appeared somewhat sickly in the early stages of growth, probably as a result of infection. The only grass which seemed to be dying in patches in consequence of attack of *Ophiobolus* was *Alopecurus pratensis*; growth was poor in the boxes of *Festuca ovina*, *F. rubra* and *F. rubra* var. *fallax*, but, in the absence of uninoculated control boxes, the poor growth of the inoculated grasses could not be definitely ascribed to infection by *Ophiobolus*. At the end of the 2 months' growing period, an individual plant from each one of the seven boxes belonging to each species was lifted for examination under the binocular dissecting microscope. An attempt was made to separate the different species into resistant and susceptible groups, according to the degree of root discoloration (Table 1).

TABLE 1. *Results of root examination under binocular dissecting microscope*

Susceptible	Resistant
<i>Alopecurus pratensis</i>	2 <i>Agrostis</i> spp.
<i>Dactylis glomerata</i>	<i>Anthoxanthum odoratum</i>
4 <i>Festuca</i> spp.	<i>Avena elatior</i>
2 <i>Lolium</i> spp.	<i>Cynosurus cristatus</i>
<i>Poa pratensis</i>	<i>Phleum pratense</i>
	<i>Poa trivialis</i>

In all species, infection of the seminal roots, as estimated by visible discoloration, was greater than that of the crown roots. The results of this examination coincided with those of previous tests made by the writer, and also in a general way with those of Padwick & Henry (1933), Russell (1934) and Winter (1939).

On 26 and 28 Sept. the tops of the grasses were cut off with scissors close to soil level,

and the sods taken out of the boxes and replaced in an inverted position. The boxes were then stacked in covered piles in the glasshouse; the weekly mean air temperature varied from 11 to 19° C., with an average of 15.4° C., during the remainder of the experiment. A sample of two boxes from each grass species at 3 and 7 weeks, and of one box at 12, 17 and 22 weeks (double these numbers were available in the fallow series), was taken for planting with test wheat seedlings; fifty-five seeds of Little Joss wheat were planted per box, in order to obtain not less than fifty seedlings. After 3 weeks' growth in the glasshouse, the wheat seedlings were washed out and examined for root infection, which was expressed as number of seminal roots infected per seedling (out of a possible maximum of six which can be produced by the seedling). Examination was made by naked eye alone of the root system floating out in water over a white background; a root was held to be infected when it showed one or more lesions with characteristic discoloration of the vascular cylinder. The end-point of infection thus selected does not always coincide with the complete

TABLE 2. *Survival of Ophiobolus graminis on roots of grasses, estimated by mean number of infected seminal roots per plant in test wheat seedlings, planted at intervals after inversion of grass sods*

	After 3 weeks	After 7 weeks	After 12 weeks	After 17 weeks	After 22 weeks	Mean in- fection figure for the 5 samples
<i>Agrostis tenuis</i>	4.76	4.69	3.77	3.40	2.78	3.88
<i>A. alba</i> var. <i>stolonifera</i>	4.50	4.54	3.79	3.08	3.43	3.87
<i>Poa pratensis</i>	4.58	4.42	3.26	2.71	1.87	3.37
<i>Festuca rubra</i> var. <i>fallax</i>	4.33	4.25	3.66	2.04	2.38	3.33
<i>F. ovina</i>	4.36	4.21	2.78	2.25	1.28	2.98
<i>Alopecurus pratensis</i>	4.44	4.08	2.00	1.34	2.13	2.80
<i>Lolium italicum</i>	4.27	3.83	2.41	2.28	—	—
<i>L. perenne</i>	4.36	3.74	2.36	1.76	1.51	2.75
<i>Dactylis glomerata</i>	4.16	4.41	2.43	1.61	0.83	2.69
<i>Cynosurus cristatus</i>	3.32	4.57	2.69	1.67	0.59	2.57
<i>Festuca rubra</i>	3.25	3.37	3.48	1.83	—	—
<i>F. pratensis</i>	3.37	3.51	2.57	1.06	0.30	2.16
<i>Anthoxanthum odoratum</i>	3.55	3.04	1.13	0.53	0.18	1.69
<i>Avena elatior</i>	1.75	2.24	1.91	0.80	0.31	1.40
<i>Poa trivialis</i>	2.53	1.94	0.38	0.27	0.04	1.03
<i>Phleum pratense</i>	2.36	0.60	0.40	0.34	0.17	0.77
Fallow	0.19	0.03	0.02	0.07	0.04	0.07

absence of *Ophiobolus* from the root system of the test wheat seedling; thus a light infection by a few hyphae may not produce a discoloured lesion visible to the naked eye. This distinction, although an important one, in no way detracts from the value of the selected end-point for its present purpose, viz. estimation of the *relative* amounts of *Ophiobolus* inoculum in the different boxes. It was unfortunate that no additional distinction could be made for this purpose between heavily and lightly infected roots, except at the expense of too much labour; as it is, the results give only a conservative estimate of the differences actually observed in degree of infection of the test wheat seedlings. The results of this first experiment are given in Table 2; the grasses are arranged in descending order of effectiveness as propagators of *Ophiobolus*.

It is impossible to draw a line at any level through this tabulation of results, such that it separates propagators from non-propagators of *Ophiobolus*. There is undoubtedly, however, a great difference between the species at either end of the Table. The results confirm the

resistance of *Phleum pratense* already reported by Padwick & Henry (1933), Russell (1934) and Winter (1939); as was to be expected, *Avena elatior* is also nearly at the bottom of the table. It is also interesting to see that the distinction made between *Poa pratensis* and *P. trivialis* in Table 1 is confirmed in Table 2. One major discrepancy between the results of the preliminary examination and those of the final test of susceptibility requires explanation, viz. the behaviour of the two *Agrostis* spp. Both in this and in previous root examinations of inoculated *Agrostis* spp. under the binocular dissecting microscope, runner hyphae were relatively scarce and discoloration only light by comparison with species of *Lolium* and *Festuca*. A clue to this paradox is possibly to be found in Winter's results. At the 38-day examination of his inoculated plants, the roots of *Agrostis alba* var. *stolonifera* showed neither browning nor external mycelium, whilst those of *A. spica-venti* exhibited occasional discoloration and sparse mycelium. After 5 months, the roots of *A. alba* var. *stolonifera* showed light browning with sparse mycelium; all plants of *A. spica-venti*, however, were now dead, presumably through infection. Furthermore, Winter had observed *A. spica-venti* ('Windhalm') to be very susceptible to infection by *Ophiobolus* in the field, stating: 'Insbesondere gibt es aber zu denken, dass Ophiobolosenester in der Regel stärker mit Windhalm verunkrautet sind, und dass dieser stets Anzeichen schweren Ophiobolosebefalls erkennen lässt. Die Pflanzen sind schwächer entwickelt, ihre Wurzeln sind zerstört, die Halmbasis ist geschwärzt, und in einzelnen Fällen treten Perithezien auf.'

Exp. II. The result of most practical interest from Exp. I was the relatively rapid disappearance of *Ophiobolus* after *Avena elatior* and *Phleum pratense*, since these species might sometimes be employed in place of the more susceptible *Lolium* spp. for temporary leys. The following series were compared in this experiment:

<i>Lolium perenne</i>	<i>Avena elatior</i>
<i>L. italicum</i>	<i>Trifolium pratense</i>
<i>Phleum pratense</i> (Scotch seed)	Fallow
<i>P. pratense</i> (Aberystwyth S. 51)	

The experiment was set up in the same manner as Exp. I, except that 120 g. of cornmeal-sand inoculum was deemed sufficient, giving a layer approximately 1 mm. in depth on the surface of the soil in each box. Another *Ophiobolus* isolate, obtained from wheat at Woburn in 1939, was used for inoculation. The boxes were inoculated and planted on 6 Sept. 1940, and this time were placed in a compartment of the glasshouse.

Approximately the same addition of nutrient solution was made to the boxes as in Exp. I. Establishment of plants was good in all boxes, but the stand was too thick in the boxes of broad red clover, which were therefore thinned out 3 weeks after planting. The growth of the tops was not as vigorous as in Exp. I, presumably owing to the poorer light conditions at this season of the year; some trouble was experienced with *Botrytis* sp. in some of the timothy boxes. After 2 months' growth, the tops of the grasses were cut off, and the sods inverted in the boxes, on 13 and 14 Nov. The root mat was by no means so strongly developed as in the boxes of Exp. I, corresponding with the poorer growth of tops. The boxes were stacked in piles in the same compartment of the glasshouse, the weekly mean air temperature of which varied from 15 to 19°C., with an average of 17.6°C., over the 4 months' sampling period. At monthly intervals from 1 to 4 months after inversion of the sods, a sample of three boxes was taken from each series; fifty-five Little Joss wheat seeds were sown per box. At 4 months, only the two *Lolium* series were sampled. The results are given in Table 3.

Comparing the results with those of Exp. I, the figures for *Phleum pratense* are seen to be closely comparable with those in Table 1. The initial infection after the two *Lolium* spp. is also of the same order as that obtained in Exp. I, but infection fell more rapidly at the latter samplings. This is probably to be attributed partly to the higher temperature of incubation of the stacked boxes and partly to the less vigorous original growth of the grasses in this experiment, leading to a smaller root mat, which was indeed observed to decompose more quickly than that obtained in Exp. I. *Avena elatior* again occupied a position intermediate between *Phleum pratense* and the two *Lolium* spp. It is interesting to note that the fungus tended to die out more rapidly under *Trifolium pratense* than in the fallow soil (cf. similar result obtained by Padwick (1935) with *Neslia paniculata*).

TABLE 3. *Survival of Ophiobolus graminis on roots of grasses, estimated by mean number of infected seminal roots per plant in test wheat seedlings, planted at intervals after inversion of grass sods*

	After 1 month	After 2 months	After 3 months	After 4 months
<i>Lolium italicum</i>	4.29	2.59	1.11	0.80
<i>L. perenne</i>	4.49	2.92	0.86	0.91
<i>Phleum pratense</i> (Scotch)	2.43	0.59	0.20	—
<i>P. pratense</i> (Aberystwyth S. 51)	2.89	0.58	0.14	—
<i>Avena elatior</i>	2.72	1.81	0.65	—
<i>Trifolium pratense</i>	0.07	0.05	0.04	—
Fallow	0.34	0.06	0.03	—

APPLICATION TO PRACTICE

The crop sequence—barley, rye-grass and clover ley, wheat—is not uncommon in English rotations; it occurs, for instance, in the traditional Norfolk four-course rotation. Since rye-grass can perpetuate *Ophiobolus*, this crop sequence must tend to perpetuate take-all, albeit at a low level, on many farms. Two courses are open to farmers on the light-textured chalk soils on which take-all is most troublesome. The first is to break up the seeds ley not later than June, and bastard fallow the land in preparation for wheat; a crop of mustard can be taken if the wheat bulb fly is considered likely to lay eggs on the fallow. The 4 months' period from breaking in June to drilling in October gives an interval in which most of the *Ophiobolus* will have died out from the infected rye-grass roots, if the fallow has been kept clean, or the growth of mustard a good one. The alternative is to replace the rye-grass component of the temporary ley by one or more of the comparatively resistant grasses, as already proposed elsewhere (Garrett, 1940). Mr William Davies, of the Welsh Plant Breeding Station at Aberystwyth, has suggested the following two seeds mixtures as suitable for special use where take-all has become a serious problem:

	Mixture 1 lb.	Mixture 2 lb.
Timothy (Scotch)	8	10
Timothy (S. 51)	4	5
Tall oat grass	6	—
Broad red clover	2	2
Late-flowering red clover	2	2
Late red clover (S. 123)	2	2
White clover (S. 100)	1	1

The value of rye-grass for temporary leys is too well established to warrant its replacement by either of these mixtures except under special circumstances. Cases have occurred,

however, notably on the Wiltshire Downs and on the Yorkshire Wolds, where yields of wheat have been so poor after rye-grass leys, whether on account of frit-fly, take-all or yet other causes, that farmers have resorted to leys of pure clover. Under such circumstances, therefore, these special seeds mixtures may find useful employment.

SUMMARY

Sixteen species of grasses were inoculated with *Ophiobolus graminis*, and their roots examined under the binocular dissecting microscope for runner hyphae and discoloured disease lesions. Whilst some species were obviously susceptible and others showed few signs of root infection, there were yet other species which were difficult to classify either as susceptible or as resistant. The effectiveness of these 16 grasses as perpetrators of *Ophiobolus* was therefore directly tested, as follows. The seed was sown in contact with a minimal amount of inoculum in boxes of a light-textured soil; 2 months after planting, the grass tops were cut off, and the sods inverted in the boxes. The degree of survival of *Ophiobolus* in the inverted sods of the different grasses was determined at approximately monthly intervals by the planting of test wheat seedlings. Whilst all 16 species propagated *Ophiobolus* to some extent, as compared with a negligible survival in fallow soil and under clover, there were notable differences in the longevity of the fungus under different grasses. The resistance of *Phleum pratense*, reported by previous investigators, was confirmed, and seeds mixtures employing this grass and *Avena elatior* in place of *Lolium* spp. were suggested for use on heavily-infected land.

I have pleasure in thanking Mr William Davies for his interest in this investigation, and for suggesting the two seeds mixtures given above; my thanks are also due to Messrs Dunns Farm Seeds Ltd., of Salisbury, and to Messrs Gartons Ltd., of Warrington, for gifts of wheat and grass seed.

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OPHIOBOLUS GRAMINIS SACC. VAR. *AVENAE* VAR.N., AS THE CAUSE OF TAKE ALL OR WHITEHEADS OF OATS IN WALES

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I. INTRODUCTION

THROUGHOUT the wheat-growing areas of the world in which the Take All disease, due to *Ophiobolus graminis* Sacc., is prevalent, the growing of oats is regarded as a valuable control measure. Where attack by *O. graminis* is the factor limiting yield, a wheat crop following oats generally gives a yield equal to or approaching that of a crop after fallow. The empirical discovery of this fact is probably due to the farmers of South Australia, where a Commission was appointed as early as 1868 to inquire into the distribution caused by the disease, though its connexion with *O. graminis* was not established by McAlpine until 1902. Rotation of wheat with oats for the control of Take All was urged by McAlpine (1904) in Victoria, by Richardson (1910) in South Australia, and by Darnell-Smith & McKinnon (1915) in New South Wales.

This conclusion has been confirmed by pot experiments under controlled conditions demonstrating the high resistance of oats to infection by *O. graminis*; such experiments were reported by Wolf & Lehman (1924) and by Kirby (1925) in the United States of America, by Schaffnit (1930) and by Müller-Kögler (1938) in Germany, by Russell (1934) in Canada, and by Föex (1935) in France. Contrary results were, however, reported by Ducomet (1913) in France and by Osborn (1919) in South Australia; both examined specimens of diseased oats carrying perithecia of *O. graminis*. Darnell-Smith & McKinnon (1915) stated that a single case of oats attacked by Take All had been recorded in New South Wales. More recently, outbreaks of Take All in oats have been reported by Gram (1929) in Jutland, and by Van Poeteren (1932) in Holland. Hynes's (1937) claim to have demonstrated susceptibility of oats to two isolates of *O. graminis* in pot tests in New South Wales is of doubtful value. He tested six isolates, incorrectly designated strains, of *O. graminis* against three varieties of wheat, two of oats, and one each of barley and rye, in pots. Two of the isolates caused some retardation in growth of the oats. As the proportion of fungus inoculum (cooked wheat and oat kernels) to

surface soil was approximately 1 : 2 by volume, a purely toxic effect of the inoculum might well have caused the stunting.

Increasing interest in Take All in wheat and barley crops in this country during the last few years has made the immunity of oats an urgent matter. Field observations, such as that by Dillon Weston (1938) in Norfolk, suggest that oats may safely be recommended as a rotation crop for control of the disease. On the other hand, recent unpublished reports of Take All affecting oat crops in Wales, communicated by Mr D. Walters Davies and Dr T. Whitehead, have been too numerous and well-authenticated to be ignored. There are other records. Massee (1912) found plate mycelium and perithecia of *O. graminis* on diseased oat plants received at Kew from Corwen in North Wales, isolated the fungus and reproduced the disease in both wheat and oat seedlings, and Jones (1926) studied the cytology of development of the perithecium of *O. graminis* in material collected on oat stubble near Aberystwyth.

The susceptibility of oats to the Take All disease in Wales might be due to at least one of three possible causes, viz. the employment of peculiar varieties of oats, breakdown in resistance of the plant owing to some unfavourable condition of the environment, or the existence of a distinct biological strain of *O. graminis*, if not of another species of *Ophiobolus*, in Wales. The experiments described below are concerned both with the properties of the Welsh isolates of *Ophiobolus* from oats, and with the resistance of oats to English isolates of *O. graminis* from wheat.

II. EXPERIMENTAL

(a) *Isolation of Ophiobolus from oats and wheat*

Three isolates of the oat-attacking *Ophiobolus* were obtained in September 1937 from infected oats collected by Miss M. D. Glynne from Pentrevoelas, Denbighshire (isolate O 3), from Caervon, Anglesea (O 4), and from Beaumaris, Anglesea (O 13). Three further isolates were obtained in the late summer of 1938 from material collected by Mr D. Walters Davies at Aberystwyth (O 20), and from further collections made by Miss Glynne in Carnarvonshire, near Bodfaen (O 21) and near Edern (O 23). English isolates of *O. graminis* were made from infected wheat collected in 1937 from Dorset (W 1), from Broadbalk field, Rothamsted (W 2), and from Mr W. Buddin's experimental plots at Reading (W 3). In 1938, two further isolates (W 4 and W 5) were obtained from one collection of infected wheat from Wareham, Dorset.

Where ripe perithecia were present on infected stubble, *Ophiobolus* was most easily isolated by arranging for the ejection of ascospores on to a sterile cover-slip suspended not more than 1 mm. above the necks of the perithecia (Samuel & Garrett, 1933). If ripe perithecia were

not present, wheat or oat grains were planted in the cavities of selected pieces of infected stubble, which were then buried in pots of moist sand. Isolations of *Ophiobolus* were more readily obtained from the young lesions on infected seedling stems than from the original infected stubble, in which other fungi were always present. The silver nitrate method of surface sterilization recommended by Davies (1935) for the isolation of *O. graminis* was employed.

(b) *Host range of Ophiobolus isolates from oats and wheat*

Experiment I. In the first glasshouse inoculation experiment, six isolates of the fungus, three from oats (O 3, O 4 and O 13) and three from wheat (W 1, W 2 and W 3), were tested against Little Joss wheat and Victory oats in seven-inch pots of sand with a nutrient solution; each series comprised twenty replicate pots. The inoculation technique used by Garrett (1936) was followed, five pre-soaked wheat or oat grains being planted in each pot over agar inoculum disks 8 mm. in diameter, cut out with a cork borer from the margin of a colony of *Ophiobolus* growing on potato dextrose agar. The oat grains were dehulled before planting to increase percentage germination. After planting, the pots were randomized on the glasshouse bench, and watered once a week with the following nutrient solution:

Calcium nitrate ($\text{Ca}(\text{NO}_3)_2$)	0.8 g.
Potassium nitrate (KNO_3)	0.3 g.
Dihydrogen potassium phosphate (KH_2PO_4)	0.2 g.
Magnesium sulphate ($\text{MgSO}_4, 7\text{H}_2\text{O}$)	0.2 g.
Potassium chloride (KCl)	0.2 g.
Ferric chloride ($\text{FeCl}_3, 6\text{H}_2\text{O}$)	0.025 g.
Distilled water	1 litre

The plants from five pots of each series were washed out at fortnightly intervals from two to eight weeks, and pickled in 60% alcohol. The three oldest roots on each plant were examined under a binocular dissecting microscope (magnification, $\times 20$) and the extent of growth of the runner hyphae measured (Garrett, 1936). The results for the first three samplings are given in Table I.

Table I. *Growth of fungus along the roots in mm.**

	W 1	W 2	W 3	O 3	O 4	O 13
After 2 weeks:						
On wheat	21 (± 1.4)	29 (± 0.7)	33 (± 1.4)	15 (± 0.6)	10 (± 0.5)	19 (± 1.0)
On oats	11 (± 1.2)	4 (± 0.3)	15 (± 1.3)	8 (± 1.0)	6 (± 0.4)	8 (± 0.3)
After 4 weeks:						
On wheat	40 (± 2.1)	49 (± 4.2)	45 (± 1.7)	43 (± 2.7)	25 (± 2.2)	55 (± 2.9)
On oats	14 (± 1.3)	23 (± 2.8)	45 (± 2.7)	33 (± 3.3)	24 (± 1.6)	14 (± 1.2)
After 6 weeks:						
On wheat	47 (± 2.0)	50 (± 2.5)	41 (± 1.9)	40 (± 2.3)	31 (± 1.7)	55 (± 2.7)
On oats	30 (± 3.6)	50 (± 7.6)	44 (± 3.4)	35 (± 2.6)	36 (± 2.9)	40 (± 2.7)

* The figures in brackets here, and in some subsequent tables, show the standard error.

The final sampling consisted of oats only; after washing out the plants, the tops were cut off about 1 cm. above the seed, and weighed.

Table II. *Weight of tops of oat plants, eight weeks old, inoculated with fungus isolates from wheat and oats*

	W 1	W 2	W 3	O 3	O 4	O 13
Mean fresh weight per plant in g.	3.4 (± 0.2)	3.4 (± 0.2)	3.6 (± 0.2)	1.4 (± 0.1)	0.8 (± 0.2)	0.5 (± 0.2)

The roots were not included in the examination, which comprised measurements both of extent of runner hyphae, and of length of root discoloured by infection; the figures are for the secondary roots formed at the first node.

Table III. *Infection of secondary roots of oat plants eight weeks old, inoculated with fungus isolates from wheat and oats*

	W 1	W 2	W 3	O 3	O 4	O 13
Mean hyphal growth in mm.	8 (± 1.3)	28 (± 6.1)	15 (± 4.3)	39 (± 5.6)	34 (± 2.9)	24 (± 5.3)
Mean length of root discoloured in mm.	1 (± 0.8)	1 (± 0.8)	1 (± 0.7)	26 (± 4.0)	28 (± 2.5)	13 (± 3.6)

The following conclusions were drawn from these results:

(1) Wheat was susceptible to isolates of *Ophiobolus* both from English wheat and from Welsh oats. The former (W 1, W 2 and W 3) produced the greater effect, many plants being killed after four to six weeks, but the type of infection and the appearance of the diseased plants were similar in all.

(2) Oats were highly resistant to the English isolates. Although runner hyphae had grown down the seminal roots for a considerable distance, the roots rarely showed any discoloration and were well developed. The secondary roots and subcoronal internode were generally free from hyphae, but in a few plants perithecia formed on the leaf bases.

(3) Oats were vigorously attacked by the Welsh isolates. O 13 was the most virulent, many plants being killed in six to eight weeks, and O 3 was the weakest. The root systems were poorly developed and severely attacked, both primary and secondary roots showing much discoloration.

Table I also shows that figures for the growth of runner hyphae of isolates from wheat and oats down the roots are not significantly different. Relying on this measurement alone, it would appear that oats are susceptible to all isolates used, but Tables II and III, giving weights of tops and extent of discoloration of the secondary roots, respectively, show that oats are seriously affected only by the isolates

from oats and are resistant to those from wheat. These observations indicate that, where different hosts are to be compared, infection is best estimated by the length of discoloured root; this figure may be supported by other data, such as the weight of tops of the plants.

Experiment II. Four varieties of oats, one of wheat, and one of barley were used in Experiment II, with the six isolates employed in Experiment I. The cereal varieties were Victory, New Abundance, and Scotch Potato white oats, Jubilee Black oats, Little Joss wheat, and Spratt Archer barley. There were thus thirty-six series, and four replicates of each were planted in ten-inch pots of sand with nutrient solution. Eight grains were sown over inoculum disks in each pot and seedlings thinned to five after ten days. After twelve weeks the tops were cut off and weighed.

Table IV. *Mean fresh weight in g. of tops of oat and wheat plants, inoculated with isolates from wheat and oats*

	W 1	W 2	W 3	O 3	O 4	O 13
Victory oats	11.8	11.4	13.4	9.3	7.6	7.7
New Abundance oats	11.0	13.7	12.4	8.2	5.0	6.8
Scotch Potato oats	12.3	11.0	10.6	7.7	7.0	9.5
Jubilee Black oats	11.7	12.0	10.2	6.2	2.5	11.1
Little Joss wheat	6.9	7.1	3.4	7.3	6.3	6.9
Spratt Archer barley	10.5	12.3	9.2	10.5	9.8	11.9

The roots were pickled in 2 % formaldehyde, and examined under the binocular microscope. The secondary roots only were considered, the primary roots having often disappeared. The total number of secondary roots, and number infected, were recorded for each plant; the percentage of infected roots for each series of twenty plants in four pots is given in Table V. These data show that the difference in

Table V. *Percentage infection of secondary roots of oat and wheat plants, inoculated with fungus isolates from wheat and oats*

Fungus isolate ...	W 1	W 2	W 3	O 3	O 4	O 13
Victory oats	6 (± 2.0)	5 (± 2.8)	9 (± 4.0)	61 (± 6.3)	91 (± 2.8)	77 (± 13.3)
Scotch Potato oats	3 (± 1.7)	0	0	72 (± 9.8)	71 (± 10.0)	74 (± 7.6)
Little Joss wheat	1 (± 1.0)	58 (± 1.7)	59 (± 6.9)	10 (± 6.1)	30 (± 15.8)	35 (± 14.1)
New Abundance oats	7 (± 2.4)	2 (± 1.7)	3 (± 2.0)	97 (± 2.0)	94 (± 3.3)	72 (± 14.3)
Jubilee Black oats	7 (± 2.6)	4 (± 2.8)	20 (± 14.9)	79 (± 11.1)	97 (± 2.4)	73 (± 5.1)
Spratt Archer barley	38 (± 13.0)	25 (± 8.8)	76 (± 1.7)	27 (± 10.5)	43 (± 10.5)	21 (± 14.0)

reaction of all four varieties of oats to the isolates from oats and to those from wheat is highly significant, thus confirming the findings of Experiment I. The differences in degree of attack on wheat and barley by the six isolates, on the other hand, scarcely appear to be significant, although a higher susceptibility of barley towards the isolates from wheat was suggested by the general appearance of the plants and the discoloration of the leaf bases.

Experiment III. This experiment was carried out in seven-inch pots of sand with a nutrient solution, later in 1938 with five new isolates, three from oats in Wales, O 20, O 21 and O 23, and two from wheat, W 4 and W 5. Three hosts were used, viz. New Abundance oats, Scotch Potato oats, and Little Joss wheat. There were thus fifteen series with six replicate pots per series; each pot was thinned to five plants. The pots were washed out after eight weeks, and the plants weighed and examined as before. The results are given in Tables VI and VII. These tables show that the new isolates behaved as those used in Experiments I and II, there being a sharp distinction in pathogenicity between the Welsh and English isolates. The three isolates from oats are seen also to differ amongst themselves, O 20 being the most virulent and O 23 attacking oats rather weakly, although its action on wheat is at least as strong as that of the other two oat isolates.

The oat isolates were again characterized by sparse development of runner hyphae on the outside of infected roots, as compared with that made by the wheat isolates. With the latter, the longitudinal extent of infection inside the root can generally be estimated by the extent of runner hyphal growth, the infection hyphae within the cells of the cortex seldom extending farther along the root than do the runner hyphae (Garrett, 1934, 1936). The isolates from oats, on the other hand, have greater powers of longitudinal spread inside the root, so that discoloration and infection may extend farther than the growth of runner hyphae on the outside of the root. Roots examined under the microscope, often showed runner hyphae entering the root and growing parallel to the surface in the subepidermal cells, cortical infection hyphae branching off in the usual way. This type of growth has been observed in wheat roots under certain conditions (Garrett, 1934), but it is much more usual in oat roots.

Experiment IV. In order to investigate further the respective host ranges of the isolates from wheat and oat, a series of nineteen common English pasture grasses was inoculated with isolates W 1, W 2, W 3, O 3, O 4 and O 13, respectively, in five-inch pots of sand with a nutrient solution. After eight weeks the extent of hyphal growth and of root discoloration was recorded, both for seminal and for crown roots. The results of this and of subsequent unpublished work, indicate that not one of these nineteen species of grass shows complete resistance even to the English isolates. In whatever way a disease rating was computed from these data, however, infection of the grasses by the Welsh isolates was both more intensive and more extensive than that by the English isolates. The complete data may be found elsewhere (Turner, 1939).

Table VII. Percentage visible infection of seminal and crown roots of oat and wheat plants, inoculated with fungus isolates from wheat and oats

Host	New Abundance oats						Scotch Potato oats						Little Joss wheat					
				W 4	W 5	O 20	O 21	O 23	W 4	W 5	O 20	O 21	O 23	W 4	W 5	O 20	O 21	O 23			
Isolate																					
Total number of seminal roots	132	141	100	122	118	144	137	134	143	143	145	146	112	133	145						
Percentage visible infection	24	12	100	83	78	23	26	100	91	66	100	100	89	78	93						
Total number of crown roots	130	137	81	145	107	210	174	184	156	177	147	148	103	133	182						
Percentage visible infection	0	0	55	28	25	0	0	53	29	2	75	68	42	24	38						

(c) *Pathological histology*

The course of infection of wheat roots by *O. graminis* has been followed in detail by Fellows (1928), Robertson (1932), and Garrett (1934). The chief characteristics of such infection are as follows: the invading mycelium is differentiated into coarse, dark-coloured runner hyphae, which grow down the outside of the roots, and more slender, hyaline infection hyphae, which branch off from these and enter the cells of the cortex. The hyphae penetrate the endodermis and invade the vascular tissues, which become severely discoloured; in these tissues, the hyphae tend to grow in a longitudinal direction. Hyphal penetration of the cell walls is frequently accompanied by the formation of highly characteristic callosities or lignitubers, first investigated by Fellows (1928). These outgrowths of the cell wall closely invest the invading hyphae, often extending some way into the cavity of the invaded cell, and sometimes appearing to prevent further growth of the enclosed hyphae. Lignitubers are frequently absent from cells invaded whilst still immature, and are generally strongest in cells infected after reaching maturity. In wheat roots eight or more weeks old, the hyphae sometimes disappear from the cells of the cortex, remaining only inside the vascular cylinder. The cortex, however, still shows obvious signs of disease, the cell walls being studded with persistent lignitubers.

The course of infection of oat and wheat roots by isolates W 4 and W 5 from wheat and isolates O 20 and O 21 from oats was followed in plants grown in sand and nutrient solution, and inoculated in the usual way under the seed; plants were washed out after ten days and subsequently at five-day intervals to fifty days. The external appearance of the plants and the extent of discoloration of the roots were noted, and the roots examined microscopically. Hand sections or whole roots were cleared in lactophenol, and stained with cotton blue. The degree of infection throughout the course of the experiment was somewhat variable in the different pots, some plants being very severely infected or completely killed after four or five weeks, while others, inoculated with the same isolate, remained almost unattacked. The more severely infected plants from all series were used for microscopical examination.

After twenty days, plants in six out of the eight series (the exception being oats inoculated with isolates W 4 and W 5, respectively) showed obvious signs of attack. The crown and leaf bases were blackened, and many of the plants were stunted. In this trial one of the isolates from oats, O 20, attacked wheat more vigorously, or at least more rapidly, than did isolates W 4 and W 5, causing very severe discoloration of the culm bases, and stunting of the root

system. At each sampling, from twenty days after planting to the final sampling after fifty days, the course of infection of wheat inoculated with all four isolates, and oats inoculated with isolates O 20 and O 21, was very similar. Penetration of the cortex had taken place in both hosts after ten days, being most evident in the proximal part of the root in contact with the inoculum disk. Hyphae were seen in the vascular tissue of wheat roots after ten days, and of oat roots after fifteen days; severe vascular discoloration was apparent after twenty days. Lignitubers were irregularly distributed throughout the infected roots of both hosts, being abundantly developed on the walls of some cells, and absent from others; their distribution could be correlated to some extent with the age of the cell when infected. The wefts of invading hyphae tended to develop into cones of mycelium, through progressive branching in the inner layers of the cortex. In older roots the lesions were no longer well defined, and infection was general throughout the cortex, though the distribution of hyphae was rather irregular. Most of the cortical cells were occupied by a few longitudinally running hyphae; here and there, however, cells were to be observed densely packed with transversely-running hyphae. Hyphae in the cortical cells of infected roots stained less deeply and became less healthy in appearance after six or seven weeks; the number of hyphae containing protoplasm steadily decreased as the plants aged. The lignitubers persisted, however, being more abundant in cells of wheat than in those of oat roots.

Infection of oat roots by the wheat isolates may now be described. In the early stages, infection followed a similar course to that by oat isolates, though less vigorous. After fifteen days, when there were abundant runner hyphae on the outside of the seminal roots, penetration had occurred at a few scattered points, and the hyphae had not penetrated inwards through more than two or three cell layers. After twenty days there were occasional lesions on the roots in which penetration was heavy and had extended to the endodermis. In a few roots, hyphae were seen at this stage inside the vascular cylinder, which showed some localized discoloration. Lignitubers were formed freely in many of the infected cells, though not in cells in the centre of lesions, and these appeared sometimes to have checked the spread of the hyphae. After twenty-seven days, the roots showed less obvious infection than after twenty days. Scattered groups of cells showed hyphae that appeared to be disintegrating, as indicated by their weakly staining properties and attenuated appearance, whilst other cells showed prominent lignitubers but no visible hyphae. Even in those taken after thirty-four and forty-one days, the roots still showed occasional localized lesions with persistent hyphae; occasionally penetration hyphae from the runner hyphae had branched in a single superficial cell until this was closely packed with stout coiled hyphae.

Amongst all samples examined from the experiment, crown root lesions were found only twice, although occasionally runner hyphae grew along the outside of these roots.

It therefore appears from these observations that the resistance of oat roots to isolates of *O. graminis* from wheat is of the chemical rather than the mechanical type (Brown, 1936).

(d) *Morphological and cultural characters of the isolates*

It was found impossible to distinguish the six Welsh isolates of *Ophiobolus*, obtained from oats, by their appearance in culture from typical isolates of *O. graminis* obtained from wheat. On the basis of colour and other colony characters on potato-dextrose agar, the isolates O 3–O 23 might all have been identified as *O. graminis*. Their growth rate was also comparable to that of isolates W 1–W 5 from wheat. A comparison of growth rate on a series of potato dextrose agars adjusted to different reactions from pH 4.0 to 9.0 showed that the oat isolates preferred if anything an initial reaction on the acid side of neutrality, whereas the wheat isolates grew best on a neutral or slightly alkaline medium. The range for suboptimal growth of both series of isolates was wide, however, and the differences were no greater than those obtained by Webb & Fellows (1926) in their study of the growth of several isolates of *O. graminis* on a number of different nutrient agars adjusted to a range of pH values.

Sharp differences were found, however, in ascospore measurements of the two series of isolates. Perithecia were obtained by Garrett's (1939) method, whereby agar-inoculated wheat seedlings were grown in boiling tubes half filled with sand plus inorganic nutrient solution, exposed to light in a north window of the laboratory. Perithecia generally matured in less than two months, on stems and especially on roots exposed to the light. The first series of tubes, containing wheat seedlings inoculated with isolates W 2, W 3, O 3, O 4 and O 13, respectively, was set up in March 1938. Measurements of the length of one hundred ascospores, taken from not less than five ripe perithecia, were made for each isolate (Table VIII).

Table VIII. *Ascospore measurements in μ*

	W 2	W 3	O 3	O 4	O 13
Mean length	86 (± 0.40)	84 (± 0.31)	116 (± 0.36)	105 (± 0.27)	110 (± 0.26)
Modal length	90	84	118	108	112
Range in length	68–104	72–102	102–130	94–130	84–130
Mean number of septa	8.5	8.0	11.0	11.5	12.5

In the second series set up in February 1939, wheat and barley seeds were inoculated with isolates W 4 and W 5, and wheat and oat

seeds with isolates O 20, O 21 and O 23, respectively. The length of one hundred ascospores, taken from not less than five perithecia, was again measured; in the O isolates, measurements of ascospores from perithecia formed on two hosts may be compared (Table IX).

Table IX. *Ascospore measurements in μ*

	W 4	W 5	O 20	O 21	O 23
On wheat	80 (± 0.35)	79 (± 0.29)	104 (± 0.42)	124 (± 0.43)	117 (± 0.40)
On oats	—	—	106 (± 0.41)	117 (± 0.38)	113 (± 0.39)

The length of the ascus for different isolates on different hosts is given in Table X, which shows that this character differs as significantly as the length of the ascospore in the two series of isolates, but there is no consistent effect of host upon the length of the ascus.

Table X. *Ascus measurements in μ*

	W 4	W 5	O 20	O 21	O 23
On barley	103 (± 0.71)	116 (± 1.0)	—	—	—
On wheat	100 (± 0.57)	112 (± 0.87)	120 (± 1.22)	138 (± 0.73)	138 (± 0.56)
On oats	—	—	128 (± 0.90)	133 (± 0.59)	131 (± 1.0)

Measurements of the length of the ascospore were also made from naturally infected material. The length of one hundred ascospores, taken from at least five perithecia, was determined from three different collections of infected oat stubble, which subsequently gave isolates O 20, O 21 and O 23, respectively, in the autumn of 1938 (Table XI).

Table XI. *Ascospore measurements in μ*

	O 20	O 21	O 23
Mean length	101 (± 0.59)	110 (± 0.52)	117 (± 0.54)
Modal length	99	115	115
Range in length	86-122	90-131	97-140
Mean no. of septa	10	12	12

STATUS OF FUNGUS FROM OATS

The isolates from oats thus differ significantly from those from wheat in the length of the ascospore, as well as in host range. The two series of isolates are, however, very similar in cultural appearance and in the symptoms produced in susceptible hosts. Miss E. M. Wakefield has kindly confirmed the difference in ascospore length, and from this and the other characteristics considers that the isolates from oats should be regarded as a new variety of *O. graminis*.

Ophiobolus graminis Sacc. var. *Avenae* E. M. Turner a typo in longioribus sporis (101-117 μ) et in planta hospitali, *Avena saliva*, differt.

SUMMARY

Outbreaks of Take All in oats have often been reported in recent years from Wales, and occasionally from Australia, Denmark and Holland, although in most parts of the world it is commonly held that oats resist the disease. Isolates of *Ophiobolus* from oats grown in Wales were indistinguishable in cultural behaviour from *O. graminis*, but they were very pathogenic to oats, which were found to be highly resistant to ordinary *O. graminis*.

This histology of the infection of oat plants by the fungus from Wales and by *O. graminis* was studied in detail. It was found that there were significant differences between the two groups of isolates in the length and septation of the ascospores, the Welsh material giving a length of 101–117 μ , the English material 79–86 μ .

The fungus from Welsh oats is therefore regarded as a new variety, *Ophiobolus graminis* Sacc. var. *Avenae* E. M. Turner.

I have much pleasure in recording my grateful thanks to Mr S. D. Garrett, who suggested this problem and supervised the work, and to Miss E. M. Wakefield for her advice on the systematic status of the isolates of *Ophiobolus* from oats.

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SOIL CONDITIONS AND THE *FUSARIUM CULMORUM*
SEEDLING BLIGHT OF WHEAT

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ATTENTION was directed to brown foot rot of cereals, caused by *Fusarium* spp., by the unusual prevalence of this disease in the southern and eastern counties in the dry season of 1938. Schaffnit (1930) in Germany and Machacek & Greaney (1935) in Canada have commented upon the association of this trouble with dry years. *Fusarium* seedling blight and foot rot appear to be of most importance in the northern counties of England, where their occurrence is perhaps to be associated with the prevalence of acid soils (Bennet, 1939); Blair (1937) demonstrated a connexion between *Fusarium* seedling blight and soil acidity by means of a field survey in New Zealand. Machacek (1936) reported an association between severe local outbreaks of *Fusarium* foot rot and excess of soluble salts in the soil in Canada. Greaney (1938) was unable to demonstrate any relation between *F. culmorum* seedling blight and deficiency of phosphate in nutrient sand cultures, but found that excess of phosphate tended to increase the severity of the disease. Machacek & Greaney (1936) showed that mechanical injury of the seed greatly predisposed seedlings to the *Fusarium* disease. Earlier experiments on the relation of soil temperature and moisture to the disease have been discussed by Garrett (1934).

The foregoing observations suggest that injury to the cereal plant, whether by drought, soil acidity, excess of soluble salts or mechanical damage to the seed, predisposes the plant to *Fusarium* infection. The search for those predisposing factors has been intensified by Sadasivan's (1939) discovery that *F. culmorum* appears to be a regularly occurring soil inhabitant, especially important in the decomposition of cereal residues, so that the occurrence of the disease can scarcely be correlated with the presence or absence of the parasite. The present paper reports experiments on the effect of soil conditions upon the seedling blight stage, and is a necessary preliminary to any study of the brown foot rot stage of the *F. culmorum* disease.

EXPERIMENTAL

The experiments were carried out in glass tumblers, using Garrett's technique (1938): Harpenden allotment soil and sand, respectively, were used for most of the experiments. Stocks of soil were air-dried, crushed, and passed through a 1.8 mm. mesh sieve; the saturation capacity of each stock, after thorough mixing, was determined by the perforated box method. In setting up an experiment, quantities of soil approximately equivalent to a volume of 180 c.c. were weighed into the tumblers, and the correct amount of water or nutrient solution necessary to bring the soil to the desired degree of saturation was added slowly to the soil in each tumbler from a measuring cylinder. For moisture contents of 50 % saturation, or more, a uniform distribution of soil moisture was thus obtained by some 48 hr. after filling. For moisture contents of less than 40 % saturation, soil and water were mixed by hand in a basin, and the moist soil then weighed into the tumblers. Seven seeds of Little Joss wheat were planted per tumbler; the method of inoculation varied with individual experiments, but after planting, seeds were covered with 40-60 g. of the same soil. By means of this variation in the weight of covering soil, and by the selection of different weight classes of tumblers for different soil

series, it was generally possible to bring all tumblers of an experiment to one of two or three final weights, which facilitated the periodical watering. The tumblers, wrapped in black paper, were randomized on the glasshouse bench, and until emergence of the seedlings were covered with glass lids lined with moist filter paper. Plants were harvested and recorded 2 to 3 weeks after planting.

In estimating the amount of disease, each plant was placed, by inspection of shoot and root system floating in water over a white ground, in one of the following seven infection classes:

Degree of infection	Numerical rating
No infection	0
Small lesions on roots or base of stem	1
Larger lesions on roots or base of stem	2
Plant yellowed and visibly stunted with severe basal lesion	3
Plant wilted and dying	4
Plant killed after emergence	5
Pre-emergence killing of seedling	6

The disease rating for any experimental series was obtained by averaging that for the individual plants comprising it, and expressed as a percentage of the maximum possible rating (6). The percentage of pre-emergence killing in any series was estimated on the basis of 94 % emergence in the stock of Little Joss seed employed throughout this work.

The isolate of *Fusarium culmorum* was originally obtained from maize, being kindly supplied to the writer by Dr F. T. Bennett. A spore suspension was always employed as inoculum in order to avoid the microbiological complications due to addition of cornmeal or other organic inocula (Garrett, 1934). The spore suspension was washed off one-month bottle slant cultures on potato-dextrose agar, and the density estimated by haemocytometer count, being subsequently adjusted by dilution if necessary. In some experimental series the seed was steeped in spore suspension before planting; in other series, 2 c.c. of the spore suspension was added to each planting hole.

(1) Concentration of spore suspension, and mode of inoculation

A stock spore suspension was made up, having a density of 267,000 spores per c.c., and diluted to $\frac{1}{10}$, $\frac{1}{100}$, and $\frac{1}{1000}$, respectively, of its original strength. Seed was used both dry and previously soaked for 48 hr. at laboratory temperature (*ca.* 16° C.); the seed was steeped in the respective spore suspensions for 20 min., and then planted immediately in sand at 50 % saturation. Results are given in Table 1.

TABLE 1. *Degree of seedling blight: concentration of spore suspension (mean of six tumblers)*

	Dry-planted seed				Germinated seed			
Thousand spores per c.c. of spore suspension	166	29.6	4	0.22	267	166	29.6	4
Disease rating	91	84	65	56	42	43	35	39

In the dry-seed series, intensity of infection is correlated with density of the spore suspension; in the pre-germinated seed series, this relation is scarcely significant, and intensity of infection is much less than that in the dry-seed series.

In a further experiment, dry seed was compared with seed pre-germinated for 42 and 66 hr. respectively, before planting in sand at 50 % saturation; inoculation was made by addition of 2 c.c. of spore suspension to each hole before planting. Results are given in Table 2.

TABLE 2. *Degree of seedling blight: dry and germinated seed (mean of six tumblers)*

Treatment of seed	Planted dry	Germinated for 42 hr. before planting	Germinated for 66 hr. before planting
Disease rating	80	49	47

The reduction in intensity of infection effected by pre-germination of the seed is once again apparent, but increase in the period of pre-soaking from 42 to 66 hr. has not reduced infection any further.

An experiment was next set up to test the effect of incubating the inoculated seed under humid conditions and at a temperature of 25° C., for varying periods before planting. A spore suspension of density 23,000 per c.c. was divided into two parts, to one of which 2 % glucose was added. Inoculation was effected by evacuating the dry seed in the spore suspension. Incubation periods of 0, 10, 20 and 30 hr. at 25° C. were employed; half the seed of each batch was planted immediately, and the other half air-dried and planted 2 weeks later. Sand at 50 % saturation was employed as the planting medium throughout. Results are given in Table 3.

TABLE 3. *Degree of seedling blight: period of incubation (mean of six tumbler)*

Incubation of inoculated seed	Spore suspension in water				Spore suspension in 2 % glucose			
	None	10 hr.	20 hr.	30 hr.	None	10 hr.	20 hr.	30 hr.
Disease rating at 1st planting	92	92	100	100	100	100	100	100
Disease rating at 2nd planting	76	59	71	87	72	36	47	82

When the seed was planted straight away, infection was very severe; some 90-100 % of seedlings were killed in every treatment. The effect of an incubation period before planting showed only in the spore suspension without glucose. When the seed was dried after the various incubation periods, and planted 2 weeks later, infection was less severe, and interesting differences were found. The intensity of infection was considerably decreased by an incubation period of 10 hr., somewhat decreased by the 20 hr. incubation, and increased only by the 30 hr. incubation period. It seems likely that the short incubation period of 10 hr. was just sufficient for germination of perhaps a majority of the spores; these might then have proved less tolerant of the sudden drying than the non-germinated spores of the no-incubation series. The 30-hr. incubation period, on the other hand, would have been sufficient for the establishment of a young mycelium, which would probably have withstood the effect of sudden drying as well as the non-germinated spores.

(2) *The limitation of seminal root infection*

An experiment was set up with the object of estimating the extension of infection by *F. culmorum* along the seminal roots under different soil conditions. Sterile sand, unsterilized sand and unsterilized allotment soil, all ± 1 % glucose and adjusted to a moisture content of 50 % saturation, were employed in this experiment. Inoculation was made by addition of 2 c.c. of a spore suspension containing 1500 spores per c.c. to each hole before planting. The degree of infection in these different series is given in Table 4.

TABLE 4. *Degree of seedling blight: length of root infected (mean of six tumbler)*

Soil type and treatment	Sterilized sand	Sterilized sand + glucose	Unsterilized sand	Unsterilized sand + glucose	Unsterilized allotment soil	Unsterilized allotment soil + glucose
Disease rating	44	65	34	23	10	6
Mean length in mm. of root infected by <i>F. culmorum</i>	10.2	32.9	2.9	7.3	1.7	0.5
No. of roots measured	149	168	136	156	164	176

Intensity of infection is greatest in the sterile sand, next in the unsterilized sand, and least in the unsterilized soil. The effect of glucose in increasing infection under sterile conditions, but decreasing infection in the unsterilized sand and soil, is by now a well-known "antibiosis" effect. Measurements were also made of the mean length of seminal root infected, from the seed downwards, in the different series. The extent of visible discoloration was measured under the binocular dissecting microscope; previous microscopical examination of cut sections had shown that the limit of infection coincided fairly closely with that of visible discoloration. These data are also given in Table 4.

It seems evident from these figures that *F. culmorum* has little power of spreading along the plant roots in the manner either of the vascular wilt *Fusaria* or of *Ophiobolus graminis*. Where extension of infection has occurred over an appreciable length of root, it can be put down to growth of the organism through the soil. This apparent inability of *Fusarium culmorum* to spread directly along the seminal roots, whether inside or outside, is of great interest, and helps to explain the limitations of its parasitism; it has been observed that if a germinating wheat seedling survives the first intensity of attack by the fungus, it frequently recovers completely, and develops into a healthy plant.

(3) Soil moisture content and infection

An experiment was set up with allotment soil and sand, respectively, adjusted to three different moisture contents, viz. 30, 50 and 80 % of saturation. Dry seed was inoculated by steeping in a spore suspension of density 2500 spores per c.c. Results are given in Table 5.

TABLE 5. *Degree of seedling blight: moisture content (mean of three tumbler)*

	In allotment soil			In sand		
Moisture content as % saturation	30	50	80	30	50	80
Disease rating	100	37	28	91	66	55

Infection was most severe at the lowest moisture content in soil, and decreased with rise in moisture content. The severe infection at low moisture content must be attributed in part to the adverse effect of low moisture upon the wheat seedling, but it must be intensified by the improved aeration conditions, which favour the fungus. It may be for this reason that rising soil moisture reduces infection to a greater extent in soil than in sand, which remains well aerated even at the higher moisture contents.

(4) Soil reaction and infection

A first experiment was carried out in sand culture at a moisture content of 50 % saturation. The nutrient solution of Gregory & Crowther (1928) was employed, different pH values being secured by the addition of sodium hydroxide and sulphuric acid, respectively. An inoculum of 2 c.c. of spore suspension of density 22,000 per c.c. was poured into each planting hole. The results are given in Table 6.

TABLE 6. *Degree of seedling blight: soil reaction (mean of six tumbler)*

Initial pH of sand	3.7	4.4	5.8	6.6	6.9	7.3	8.1
Final pH	3.9	5.7	6.1	6.1	7.3	7.4	7.8
Disease rating	81	93	93	64	59	48	87

Intensity of infection was lowest at pH 7.3, and increased with acidity of the sand culture to pH 4.4; with a further increase of acidity to pH 3.7, intensity of infection declined somewhat. On the other hand, severity of infection also increased markedly with rise in pH value to 8.1.

In a second experiment, different reactions were obtained by addition of sodium hydroxide and sulphuric acid, respectively, to allotment soil, which was then incubated at a moisture content of 50% saturation in loosely covered flower pots in the glasshouse for 2 weeks. The treated soils were then dried, crushed and sieved, in preparation for use in the tumblers. The experiment was conducted at the usual soil moisture content of 50% saturation; inoculation was effected by steeping the dry seed in a spore suspension of density 12,500 per c.c. Results are given in Table 7.

TABLE 7. *Degree of seedling blight: soil reaction (mean of three tumblers)*

	+1.8%	+1.3%	+0.8%	No	+0.3%	+0.6%	+1.2%
	H ₂ SO ₄	H ₂ SO ₄	H ₂ SO ₄	treatment	NaOH	NaOH	NaOH
Initial pH	4.4	5.7	6.4	7.1	7.3	7.9	8.1
Final pH	4.9	5.9	6.2	6.7	7.1	7.8	8.1
Disease rating	55	47	23	42	34	43	74

Intensity of infection in this experiment was lowest at pH 6.4 and increased on the one hand with acidity to pH 4.4, and on the other with alkalinity to pH 8.1.

It seemed possible, in view of the absence of any reported association between *F. culmorum* seedling blight and alkaline soils in the field, that the sodium hydroxide might be exerting a directly injurious effect upon the wheat seedling, and hence predisposing it to infection. In a third experiment, therefore, calcium hydroxide was compared with sodium hydroxide. The air-dry untreated allotment soil was weighed directly into the tumblers, and the requisite amounts of sodium hydroxide and sulphuric acid, respectively, added in solution. An additional series of tumblers was filled with soil previously mixed dry with finely divided calcium hydroxide. Soil moisture content was adjusted to 60% saturation, and all tumblers incubated in the laboratory for 1 week before planting. The dry seed was inoculated by steeping in a spore suspension of density 9000 per c.c. Results are given in Table 8.

TABLE 8. *Degree of seedling blight: soil reaction (mean of six tumblers)*

	+0.8%	+0.6%	+0.3%	No	+0.5%	+0.5%
	H ₂ SO ₄	H ₂ SO ₄	H ₂ SO ₄	treatment	NaOH	Ca(OH) ₂
Initial pH	3.7	3.9	5.0	6.9	8.4	8.5
Disease rating	55	42	31	23	91	23

Whereas infection was higher in the sodium hydroxide series than in any other, infection in the calcium hydroxide series was no higher than that in the untreated soil. The growth of the control plants in the sodium hydroxide series in this experiment was very poor indeed; 18% of the plants died, and the non-inoculated controls received a disease rating of 67, as compared with a rating of 4 for the control plants both in the untreated and in the limed soil. In the preceding experiment, conditions would have permitted greater reaction between the sodium hydroxide and the soil before the start of the experiment, and hence a smaller injurious effect on the seedlings.

The results of these three experiments therefore support the field observations of Blair (1937) as to the prevalence of *Fusarium* seedling blight on the more acid soils in New Zealand.

(5) *Plant nutrition and infection*

The effect of plant nutrition upon infection was investigated in sand culture. Acid-treated and washed sand was brought to a moisture content of 50% saturation by addition of the nutrient solution of Gregory & Crowther (1928), which was used, however, at double strength, viz.:

NaNO ₃	3.04 g./l.	MgSO ₄ ·7H ₂ O	0.42 g./l.
Na ₂ HPO ₄ ·12H ₂ O	1.0 „	Boric acid	0.002 „
K ₂ SO ₄	0.62 „	MnSO ₄	0.002 „
CaCl ₂	0.16 „	Fe-tartrate	0.28 „

The manurial treatments comprised full nutrient solution, no nutrients, and elements nitrogen, phosphorus, and potassium singly and in pairs, giving presence and absence of N, P, and K. For inoculation, 2 c.c. of a spore suspension, of density 18,000 per c.c., was added to each planting hole. In order the better to demonstrate any effect of the nutrients upon seedling resistance, pre-germinated seed was planted, to reduce killing of the seedlings to a minimum (see Tables 1 and 2). The degree of replication was increased from the usual six to ten tumblers per series in this experiment, making seventy plants for each treatment. Results are given in Table 9.

TABLE 9. *Degree of seedling blight: differential nutrition (mean of ten tumblers)*

	Nil	N	P	K	NP	NK	PK	NPK
% pre-emergence killing	1	0	3	1	6	3	0	6
% post-emergence killing	29	0	4	23	3	1	17	0
Disease rating, excluding pre-emergence killing	57	33	41	55	34	37	46	33

Intensity of infection is highest in the no-nutrient series, and lowest in that receiving full nutrients. Addition of N, either alone or in combination with P or K, seems to be almost or quite as effective in reducing infection as the full nutrient solution. P, either alone or in combination with K, is markedly less effective in reducing infection than is N. K alone has not significantly reduced infection below that in the no-nutrient series; there is a suggestion that in combination with N and P, respectively, it actually increases infection above that occurring with either N or P alone.

SUMMARY

The influence of soil conditions upon the *Fusarium culmorum* seedling blight of wheat has been studied by means of the glass tumbler technique. Intensity of infection increased with density of the spore suspension used to inoculate the seed, but was reduced by germinating the seed for 48 hr. or more before inoculation. Infection was most severe at a low soil moisture content and in acid soil. In nutrient sand culture, infection was highest in the no-nutrient series and lowest in that receiving full nutrients. Nitrogen alone appeared to be as effective in reducing infection as the full nutrient solution, phosphorus was less effective, whilst potassium had no beneficial effect at all upon seedling resistance.

This work was commenced in the Department of Plant Pathology, Imperial College of Science and Technology, and continued at the Rothamsted Experimental Station. The writer is indebted to Prof. W. Brown and Mr S. D. Garrett, respectively, for suggesting the problem, and for advice and direction during the progress of the investigation.

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THE COLONIZATION OF BURIED WHEAT STRAW BY SOIL FUNGI, WITH SPECIAL REFERENCE TO *FUSARIUM CULMORUM*

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(With 11 Text-figures)

THE saprophytic life of *Fusarium culmorum* has, until recently, been little studied, although its association with brown foot rot of cereals in both the seedling blight and whiteheads condition has long been established. *F. culmorum* has also been found in association with *Ophiobolus graminis* on plants attacked by the latter organism (Bennett, 1928; Schaffnit, 1930; Guyot, 1934; Sadasivan, 1939). More recently the presence of *Fusarium culmorum* on the roots and crowns of apparently healthy wheat plants has been recorded by Samuel & Greaney (1937), who isolated the fungus from surface-sterilized roots and crown pieces of normal plants which showed no disease symptoms. Three fields in different localities were sampled at fortnightly intervals between heading and harvest; in two of the fields the percentage of *F. culmorum* isolated increased with advance of the season, reaching a maximum in the isolations made from the stubble after harvest. This called attention to the earlier work of Broadfoot (1934), who obtained 20-60% of *F. culmorum* in isolations from many thousands of wheat plants taken at random from a series of rotation plots. Samuel & Greaney concluded that the fungus must have been present in the soil and entered the root only as vitality was lost after flowering, causing no appreciable damage, and that there was a further development of the fungus on the stubble after the crop had been cut.

Sadasivan (1939), who reviewed previous investigations on *F. culmorum*, studied the succession of soil fungi colonizing buried wheat straw. Natural unsterilized wheat straws, and sterilized straws treated with a 2% solution of sodium nitrate, were buried in a series of soils of various types. Samples of the straws were taken at regular intervals, surface-sterilized and plated out on potato-dextrose agar. Sadasivan found that *F. culmorum* developed on straws taken from all the soils examined and that *F. culmorum*, *Penicillium* spp. and *Mucor* spp. were numerically the most important soil fungi colonizing the straws. *Fusarium culmorum* and *Mucor* spp. were dominant in the early stages of colonization, being replaced by *Penicillium* spp. in the later stages of decomposition; the previous sterilization and nitrogenous treatment of the straws appeared to favour the development of *Penicillium* spp. at the expense of *Fusarium culmorum* and *Mucor* spp. Isolates of *Fusarium culmorum* thus obtained as saprophytes on wheat straw were shown to be pathogenic to wheat seedlings. On the basis of these observations, Sadasivan provisionally placed *F. culmorum* in Reinking & Manns' (1933, 1934) group of *soil inhabitants*, or *true soil fungi*.

The work described here was undertaken to confirm Sadasivan's conclusions concerning the saprophytic activity of *F. culmorum* and to find out whether the colonizing activity of

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F. culmorum and other soil fungi fluctuated with the season. Sadasivan observed that soils collected in the autumn tended to give a higher proportion of *F. culmorum* and *Mucor* spp., and a lower proportion of *Penicillium* spp., than those collected in the spring. This suggested that *Fusarium culmorum* might reach a peak of activity in the soil in the autumn, after the ploughing in of the cereal stubble, and that later on in the following spring this fungus might be replaced, both in the straw and in the adjacent soil, by *Penicillium* spp.

EXPERIMENTAL

(a) Colonization of wheat straw by soil fungi

Experimental methods. The technique employed in these experiments on the fungal colonization of buried straw was essentially the same throughout, and hence a general description will serve, with modifications, for all the experiments to be described. The method used by Sadasivan (1939) was followed, sterilized straw being used for all experiments. The straw was selected from the internodes of healthy wheat plants and cut into pieces about 1 in. long. After soaking in tap water for 18 hr. the straws were drained and autoclaved for 30 min. at 1 atm. The straws were potted immediately after collection of the soil from the field, with as little air drying of the soil as possible. Sterilized linen bags were used for the collection of the soils, which, while in the bags, were broken up and well mixed, the larger stones and pieces of plant material being removed from each sample. The autoclaved straws were buried in between layers of soil in 3½ in. flower pots (size 60). In Exp. 1 only 12 straws, in 3 layers of 4, were buried in each pot; in the remaining experiments 25 straws, in 5 layers of 5, were buried per pot. The pots were watered after filling, and stood over a layer of moist sand in a lightly covered wooden box in the laboratory, being subsequently watered as necessary. The temperature of the laboratory seldom varied outside the range of 15–20° C., and the weekly mean temperatures were always within this range.

After the requisite period of incubation a sample of pots from each experimental series was taken and the straws washed over a sieve. The straws from each pot were kept together and surface-sterilized by shaking with an appropriate reagent in a rubber-stoppered tube, followed by washing in four changes of sterilized tap water. The straws were plated in the pure culture room on acidified (pH 5.0) potato-dextrose agar, four straws to each plate. The plates were incubated at 25° C., and the fungal colonies developing from each straw over a period of 14 days recorded. Frequently more than one and sometimes up to five colonies developed from each straw.

To simplify the presentation of data, only the numerically dominant species and genera have been tabulated; these are *Fusarium culmorum*, *Penicillium* spp., *Mucor* spp. and *Trichoderma* spp. Remaining fungal colonies were recorded as 'other fungi'. Results have been expressed graphically throughout. *The number of colonies belonging to each of the four groups has been expressed as a percentage of the total number of straws plated out*; the number of straws giving one or more fungus colonies on the plate has also been expressed separately.

In some samplings, plates were overrun by *Rhizopus* contaminations; such plates had generally to be discarded, which in effect reduced the size of the sample of straws taken. This *Rhizopus* contamination was found to be particularly troublesome in August, both in 1939 and 1940.

Exp. 1. In this experiment soil samples were taken systematically from nine of the plots and fields on the Experimental Station farm in each month of the year (1938–9) except January. The Rothamsted soil is a flinty clay loam; the nine different experimental plots or fields selected for sampling were as follows: Agdell Plot 1 (wheat after fallow in four-course rotation); Agdell Plot 2 (wheat after clover in four-course rotation); Great Harpenden, west half (wheat after beans); Great Harpenden, east half (wheat after clover); Broadbalk four plots (fallow after 4 years wheat and wheat after 1 year fallow, respectively); 86 lb. nitrogen/acre applied as sulphate of ammonia and as sodium nitrate, respectively), and Stackyard (pasture). The soil samples were taken at the mid-period of each month from September 1938 to September 1939 inclusive; the batch of plates from the August 1939 sampling was spoilt by contamination with *Rhizopus*.

The method of sampling was as follows: a convenient sampling line was adopted for each field or plot at the first sampling and adhered to at subsequent monthly samplings. The first sample was taken six paces into the field or plot, and the other samples at six-pace intervals along the line. The soil samples were therefore taken from approximately the same sites at each sampling. Ten samples

were taken per plot; each sample, comprised of four trowels full of soil taken at arm's length to the front, right, rear and left, respectively, was placed in a sterilized bag. The trowel was not sterilized but was merely wiped clean between the taking of consecutive samples.

The samples were potted separately, and the pots incubated for 14 days before the washing out and plating of the straws. Surface sterilization was effected in this experiment by shaking for 1-1½ min. in 1 in 1000 aqueous mercuric chloride, followed by washing in four changes of sterile tap water.

Fig. 1 shows the relationship of the different groups of fungi to one another when the results from all nine sampling areas are averaged. *Fusarium culmorum* was the numerically dominant organism colonizing the straws throughout the year, followed by the group of *Penicillium* spp., whilst *Mucor* spp. and *Trichoderma* spp. remained at a constantly lower level than the first-named through most of the year. The proportion of *Fusarium culmorum* colonies fluctuated, but it is difficult to attach any special significance to these fluctuations; the same is true of the relative numbers of *Trichoderma* spp., which remained at a lower level throughout. The proportion of *Mucor* colonies was higher in the two September samplings than in any other month, whilst the proportion of *Penicillium* was lowest in the two September samplings.

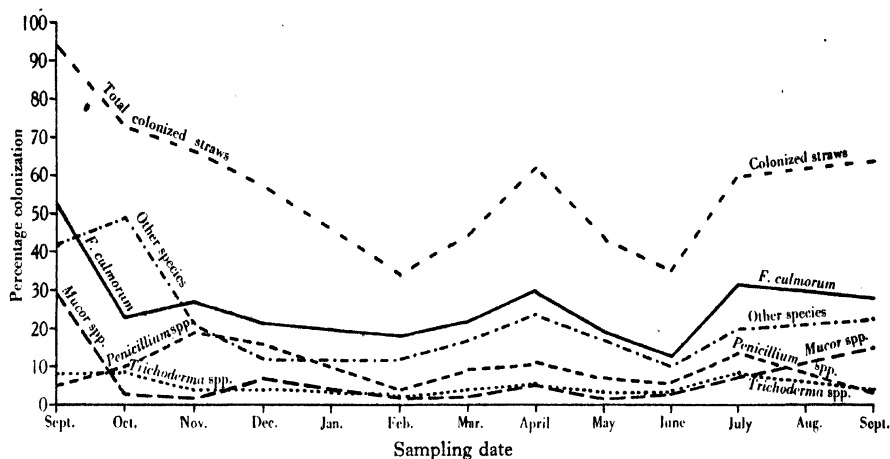


Fig. 1. Development of fungi from straws buried in the nine soils of Exp. 1.

Fig. 1 also shows the percentage of straws yielding fungus colonies throughout the year as a mean of all plots; this affords a measure of fungus activity in straw colonization. As might have been expected, the curve falls to a minimum in February and rises to a second maximum in September. An unexpected depression occurs at the May and June samplings; this may be due to the heavy rain occurring just before sampling days in these two months, which led to a high soil moisture content, even reaching saturation in some plots.

The variation in fungal colonization of the straws in the different sampling areas is shown in Fig. 2, in which the figures for the whole sampling period are averaged for each area. *Fusarium culmorum* was highest in Stackyard (grass) and in Plot 16 (wheat and fallow, sulphate of ammonia) of Broadbalk, next in Plot 7 (wheat and fallow, nitrate of soda) of Broadbalk and in Great Harpenden (wheat after beans and clover respectively) and lowest in Agdell (wheat after fallow and clover, four-course rotation). The numbers of this fungus did not appear to be influenced by the fallowing policy on Broadbalk, but were affected by

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the form of nitrogen applied. *Penicillium* spp. was the group next in importance to *Fusarium culmorum*, but numbers did not vary much from field to field. *Mucor* spp. and *Trichoderma* spp. were the least important, except in Agdell field under the four-course rotation, in which all four groups were equally important.

The pathogenicity of sixty-five isolates of *Fusarium culmorum* obtained in this experiment at different times of the year and from the nine different sampling areas was tested on wheat seedlings in pots of sand. The pathogenicity of these isolates, estimated by Shen's (1940) disease rating, compared favourably with that of two isolates of *F. culmorum* obtained from diseased plants, which were included in the test.

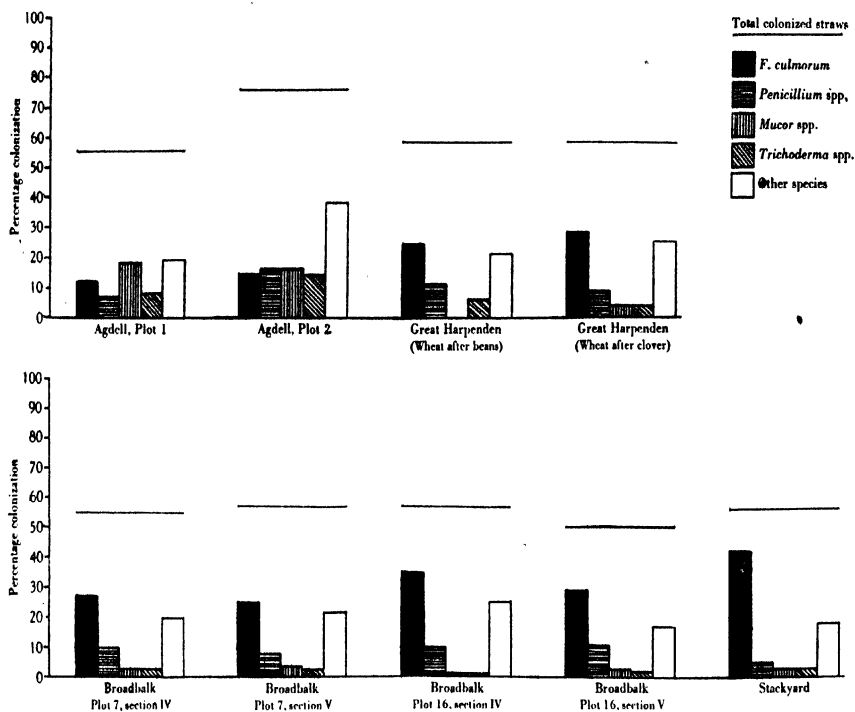


Fig. 2. Development of fungi from straws buried in soil from the nine sampling areas of Exp. 1, averaged for each area for the whole sampling period.

Exp. 2. In this experiment, the buried straw technique was employed with sixteen different soils collected from widely separated parts of the country. The soils were collected in the early part of August 1939 and stored in the sample bags until the experiment was set up in October. The different soils are described below, pH values being determined by the quinhydrone electrode:

- (1) *Helpston*. A medium loam collected in Northamptonshire, pH 8.01. Wheat 1938-9.
- (2) *Newark*. Clay-loam collected near Peterborough, pH 6.32. Wheat 1938-9.
- (3) *Crowland*. Black fen soil from Lincolnshire, pH 7.30. Wheat 1938-9.
- (4) *Normanton*. Loam from Rutland, pH 7.89. Wheat 1938-9.
- (5) *Beenham*. Loam, overlying the chalk, from Berkshire, pH 6.87.
- (6) *Lambourn*. Loam, overlying the chalk, from Berkshire, pH 7.66. Oats 1938-9.
- (7) *Cholsey*. Loam, overlying the chalk, from Berkshire, pH 7.66. Wheat 1938-9.
- (8) *Lockford*. Loam, overlying the chalk, from Hampshire, pH 7.87. Wheat 1938-9.
- (9) *Cambridge*. Calcareous loam from Cambridge University Farm, pH 7.94. Wheat 1938-9, undersown with clover.

- (10) *Duxford*. Calcareous loam from Cambridgeshire, pH 7.87. Wheat 1938-9.
- (11) *Knebworth*. Clay-loam from Hertfordshire, pH 7.61. Wheat 1938-9.
- (12) *Brynamlwg*. Medium loam from Cardiganshire, pH 5.47. Wheat 1938-9.
- (13) *Nancellan*. Clay-loam from Cardiganshire, pH 6.52. Oats 1938-9.
- (14) *Nanllan*. Clay-loam from Cardiganshire, pH 6.09. Wheat 1938-9.
- (15) *Cwm I*. Loam over shale from Cardiganshire, pH 5.27. Oats 1938-9.
- (16) *Cwm II*. Loam over shale from Cardiganshire, pH 5.51, collected from the vicinity of Cwm I but ploughed up from waste land. July 1939.

Four pots were filled from each sample of soil; twenty-five sterilized straws being buried in each, and the pots incubated for 28 days. In this experiment the washed straws were surface-sterilized by shaking for 1-1½ min. in 1 in 1000 aqueous mercuric chloride and then plated out as usual on acidified potato-dextrose agar.

The results are given in Fig. 3; the different soils are arranged in order of decreasing importance of *F. culmorum*, which was dominant in only four soils, viz. Newark, Normanton, Helpston and Crowland. In the other twelve soils, *Penicillium* spp. was the dominant group of fungi. The inverse relationship between *Fusarium culmorum* and *Penicillium* spp. is the most interesting feature of this experiment. Too much importance should not be attached to differences in straw colonization in these sixteen soils, on account of differences in soil moisture content at the time of collection, and also in the rate of drying out of the soil in the bags. Fig. 3 also shows that in no soil did the percentage of straws colonized fall below 80. The number of genera of fungi identified on the straws in this experiment was meagre; *Acrostalagmus* spp. were found on the straws from thirteen soils, *Aspergillus* spp. from three soils, *Fusarium* spp., other than *Fusarium culmorum*, from three soils, *Gliocladium* spp. from five soils, *Stemphyllium* sp. from one soil, and a *Pythium*(?) sp. from four soils. Many of the 'other fungi' had to remain classed as 'sterile mycelia'.

(b) Pathogenicity test of *F. culmorum* isolates from Exp. 2

Isolates obtained in this experiment from eleven of the sixteen sterilized soils were tested for pathogenicity against six isolates of the fungus obtained from diseased cereal plants, viz.:






Isolate P.A. from oats.	Isolate P.D. from barley.
Isolate P.B. from wheat.	Isolate P.E. from wheat.
Isolate P.C. from maize.	Isolate P.F. from wheat.

In this test unsoaked Little Joss wheat seed was inoculated by steeping for 20 min. in a spore suspension of 20,000 per c.c.; the suspensions were obtained from 10 weeks old bottle slants of the different isolates on potato-dextrose agar, and were all adjusted to the above spore density. Seven inoculated seeds were planted in each pot of silver sand, five pots being allotted to each isolate. A control series of sixteen non-inoculated pots was planted at the same time. Before planting the pots of sand were watered once with nutrient solution. The experiment was carried out in the glass-house in March 1940; the plants were washed out and the degree of infection recorded by Shen's (1940) method after 21 days (Table 1).

The percentage emergence in the sixteen control pots was 95; only 5/112 plants showed any root infection and the disease rating of these control plants was only 0.8. The results of this experiment show once more that the 'saprophytic' isolates of *Fusarium culmorum* from buried straws are no less pathogenic to wheat seedlings than isolates obtained directly from diseased cereal plants.

Exp. 3. In the preceding experiments a negative correlation between the occurrence of *F. culmorum* and that of *Penicillium* spp. in the different soils was suggested (Fig. 3);

Total colonized straws

-  *F. culmorum*
-  *Penicillium* spp.
-  *Mucor* spp.
-  *Trichoderma* spp.
-  Other species

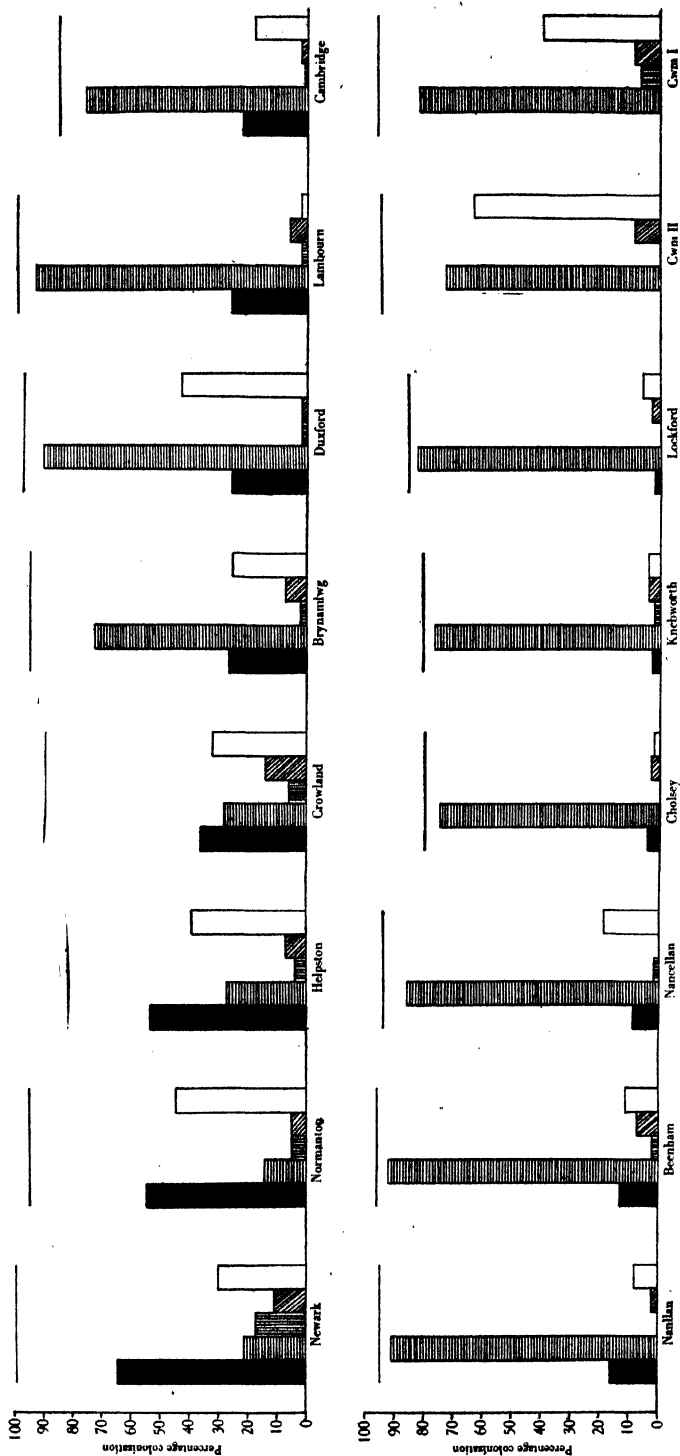


Fig. 3. Development of fungi from straws buried in the sixteen soils of Exp. 2.

confirmation on a more extensive scale of Sadasivan's observations on the replacement of *Fusarium culmorum* by *Penicillium* spp. in the buried straws at a later stage was felt to be needed. Accordingly an experiment on the succession of fungi in straws buried in three different soils was planned, the samplings to be spread over a period of some 6 months.

TABLE 1. *Infection of wheat seedlings by isolates of Fusarium culmorum from Exp. 2*

Isolate	Infected seedlings	Pre-emergence killing	Post-emergence killing	Disease rating
Helpston	23	2	1	27
Crowland	25	1	1	24
Newark	20	2	0	20
Normanton	28	1	2	30
Beenham	20	1	0	18
Lambourn	24	2	2	26
Brynamlwg	17	1	1	17
Nancellan	15	2	1	19
Cambridge	24	4	1	30
Duxford	21	3	2	28
Nanllan	21	4	5	26
P.A.	19	2	1	23
P.B.	24	2	0	21
P.C.	29	3	2	36
P.D.	23	0	0	22
P.E.	22	3	1	22
P.F.	28	3	3	36

The soils were as follows:

(1) *Great Harpenden*, as in Exp. 1; the soil was collected from over the ploughed stubble of the previous season's (1939) wheat crop; the field had carried clover in 1938.

(2) *Harpenden allotment soil*, as used by Sadasivan in his experiments, but not from the identical site; collected from under weeds.

(3) *Woburn soil*, obtained from one of the arable fields at the Woburn Experimental Station.

All soils were collected in March 1940, immediately before the setting up of the experiment, and were passed through a $\frac{1}{4}$ in. sieve. As internodal straws disappear after less than 6 months in the soil, nodal straw of the type used by Garrett (1938) was employed. Twenty-five straws were buried in each pot; eight pots of each soil, giving 200 straws in all, were taken at every sampling. After washing, the straws were surface-sterilized by shaking for $1\frac{1}{2}$ min. in 1 in 1000 aqueous mercuric chloride. Samples were taken at 2 weeks, 4 weeks and thence at 4 weekly intervals to 20 weeks. Results are shown in Figs. 4-6.

The percentage of straws colonized by fungi in the three soils was consistently highest throughout the experiment in Great Harpenden soil and almost as consistently the lowest in Woburn.

Considering the results from the three soils together, Sadasivan's observations as to the paramount importance of *Fusarium culmorum* in the early, and *Penicillium* spp. in the later, stages of straw colonization were confirmed only in the Great Harpenden and Woburn soils. Allotment soil has given a different result in this experiment, although Sadasivan recorded 100% of *Fusarium culmorum* from this type of soil in one of his samplings. In all three soils of this experiment, *Mucor* spp. have fairly consistently declined from a maximum at the first and second samplings, whilst *Trichoderma* spp. have fluctuated but shown no very pronounced trend.

Exp. 4. The results of the first three experiments suggested that the type as well as the extent of fungal colonization might be influenced by the surface-sterilizing reagent employed, and by the time of treatment given. It was also possible that the type of colonization might

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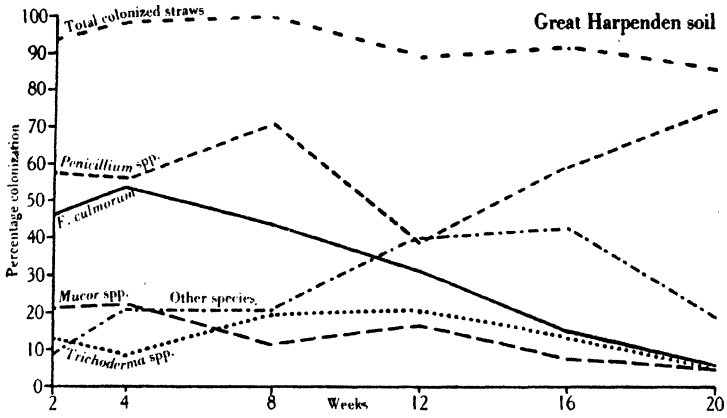


Fig. 4. Development of fungi from straws buried in Great Harpenden soil of Exp. 3.

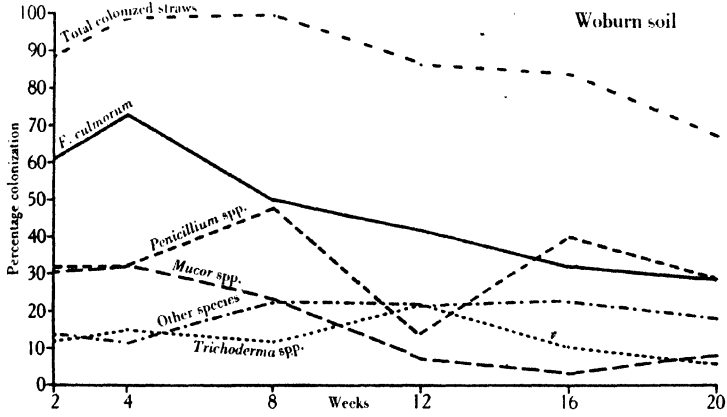


Fig. 5. Development of fungi from straws buried in Woburn soil of Exp. 3.

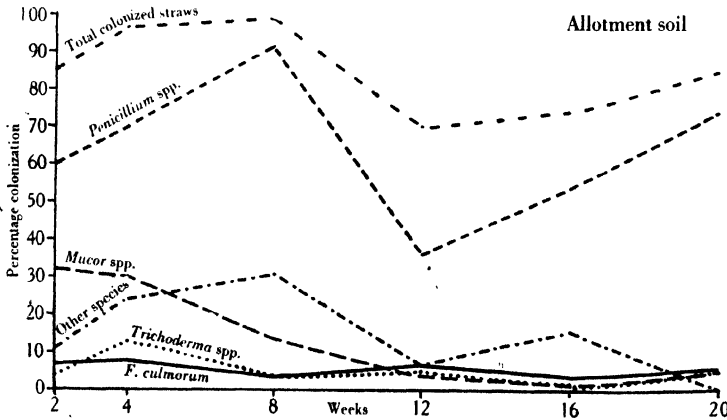


Fig. 6. Development of fungi from straws buried in allotment soil of Exp. 3.

be influenced by the culture medium employed and that a medium other than acidified potato-dextrose agar might give somewhat different results. In Exps. 1 and 2, the time of immersion in the 1 in 1000 mercuric chloride solution varied between 1 and 2 min., and it was thought that part of the inconsistencies in the results might be traced to this cause. In Exp. 3 the time of immersion in mercuric chloride was fixed as rigidly as possible at $1\frac{1}{2}$ min.

Different surface-sterilizing agents were compared by Mead (1933), both for the isolation of fungi from wheat roots, and for the production of sterile wheat seedlings by seed sterilization. Silver nitrate was found most satisfactory for seed sterilization, but its action was too strong for the isolation of pathogenic fungi; mercuric chloride proved as good for the first purpose, and more satisfactory for the second. Calcium hypochlorite was very satisfactory for root isolation, but for seed sterilization a long period of immersion was required. Hydrogen peroxide was only a mildly toxic agent and its strength soon depreciated. Washing with sterile water in Simmonds' (1930) washing machine was the most satisfactory method of treating young and delicate roots from which fungal isolations were required.

Davies (1935) compared mercuric chloride, silver nitrate, calcium hypochlorite and hydrogen peroxide at different concentrations and for different times of immersion as surface-sterilizing agents for the isolation of *Ophiobolus graminis* from young infected wheat stems. Silver nitrate was less toxic to *O. graminis* than mercuric chloride, and the frequency of isolation of this fungus was substantially higher with the first named sterilizing agent. With *Helminthosporium sativum*, on the other hand, silver nitrate was more toxic than mercuric chloride. These observations of Davies are of particular interest, inasmuch as they afford evidence of specificity in the action of sterilizing agents on these root infecting fungi.

The sterilizing agents used in the experiment described here were mercuric chloride, calcium hypochlorite and silver nitrate, which were employed as follows:

Mercuric chloride—as a 1 in 1000 aqueous solution for 2 and 4 min. periods of immersion, respectively, followed by washing in four changes of sterile tap water. This agent at 1 in 1000 strength was employed both by Mead (1933) and by Davies (1935).

Calcium hypochlorite—as a 1 in 14 suspension for 10 and 20 min. periods of immersion, respectively, followed by washing in six changes of sterile tap water. Mead and Davies both used a calcium hypochlorite suspension of the 1 in 14 strength, the former using a 20 min. period, and the latter a 2 and 5 min. period of immersion respectively.

Silver nitrate—the technique of Padwick (1938) was followed. Periods of 2 and 4 min., respectively, in 1 % silver nitrate were followed by a brief immersion in saturated sodium chloride solution, followed by immediate plating out of the material on agar. The saturated sodium chloride was not heat sterilized but was prepared some time before use. In this method, the sterilizing agent, silver nitrate, is not washed away by sterile water, but is precipitated in the saturated sodium chloride solution as silver chloride, which is highly insoluble in water.

In setting up this experiment, the straws were buried in Great Harpenden soil, as collected in March 1940 for Exp. 3, with twenty-five straws per pot. Two samplings were made at 14 and 28 days, respectively, and at each sampling four pots (100 straws) were allotted to each of the six sterilization treatments. As usual, acidified potato-dextrose agar was used as the plating medium. The results of this experiment are given in Figs. 7 and 8.

Percentage colonization of the straws was greater after 28 days than after 14 days; at both samplings it was highest in the calcium hypochlorite series. At the 28-day sampling, the percentage colonization in the 2 min. silver nitrate series was lower than that in the 4 min. series.

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Fusarium culmorum was the dominant colonist in the calcium hypochlorite series, both in the 10 and 20 min. treatments and at both 14- and 28-day samplings. Its percentage occurrence was depressed by the 2 min. treatments with silver nitrate and with mercuric chloride, respectively, and it was completely cut out by the 4 min. treatments with these two agents, except for a 2% occurrence in the 4 min. mercuric chloride treatment at 28 days.

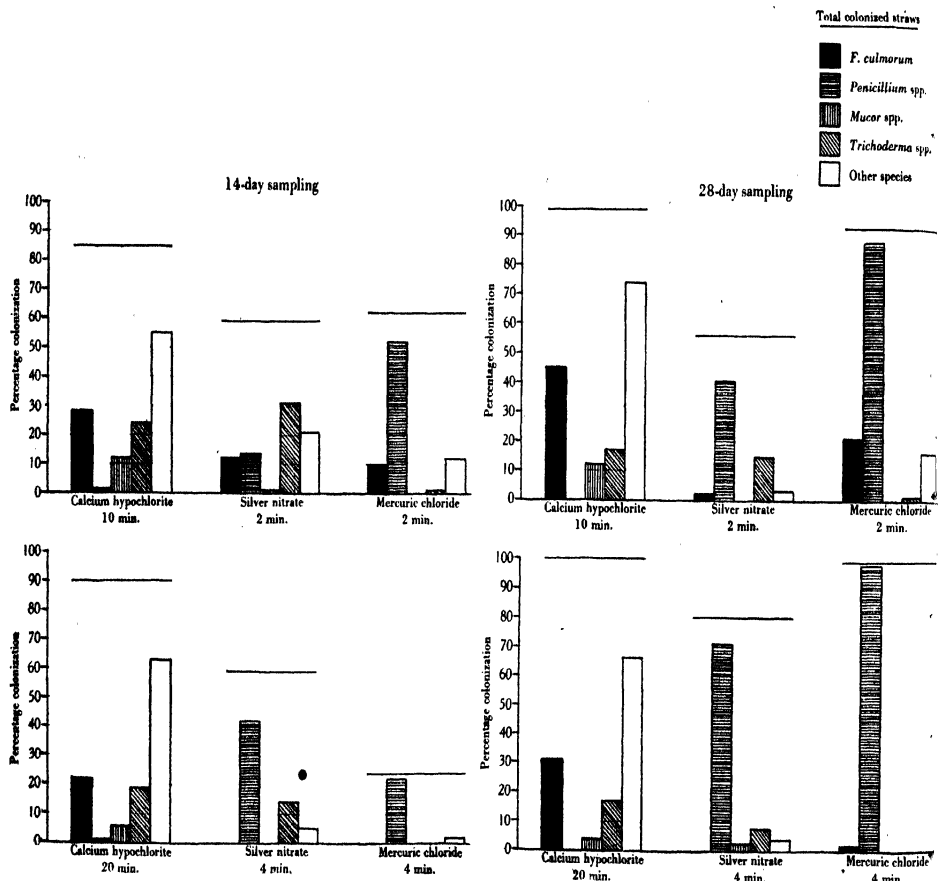


Fig. 7. Development of fungi from straws buried for 14 days in soil of Exp. 4, after treatment with different sterilizing agents.

Fig. 8. Development of fungi from straws buried for 28 days in soil of Exp. 4, after treatment with different sterilizing agents.

Penicillium spp. were reduced to 1-2% occurrence by the calcium hypochlorite treatments at 14 days and to nil at 28 days. They were the dominant group in the silver nitrate and mercuric chloride treatments at both strengths and at both samplings with one exception, the 2 min. silver nitrate at 14 days, in which *Trichoderma* spp. were dominant. The relative ascendancy of *Penicillium* spp. was greater in the mercuric chloride than in the silver nitrate treatment and increased with the time of treatment from 2 to 4 min.

Mucor spp. were highest at the 10 min. treatment with calcium hypochlorite, were lower

in the 20 min. treatment with this agent, occurred only twice, at and below the 3 % level, in the silver nitrate series, and not at all in the mercuric chloride series.

Trichoderma spp. appeared to be particularly tolerant of silver nitrate and were the dominant fungi in the 2 min. treatment at 14 days. The 4 min. treatment with this agent, however, suppressed *Trichoderma* spp. more than the calcium hypochlorite treatments, in which percentage occurrence of *Trichoderma* spp. was second only to *Fusarium culmorum* throughout both treatments and samplings. Mercuric chloride appeared to be particularly toxic towards *Trichoderma* spp., reducing them to a 2 % occurrence at the 2 min. treatment and to nil at the 4 min. treatment at both samplings.

Thus, calcium hypochlorite secured the most even distribution of colonies amongst the four groups, with *Fusarium culmorum* as a dominant; silver nitrate favoured the dominance of *Penicillium* spp., with *Trichoderma* spp. markedly subdominant; mercuric chloride greatly simplified the fungus flora of the plates, which consisted in the 2 min. treatment largely, and in the 4 min. treatment almost entirely, of *Penicillium* spp. The results are obviously not to be interpreted on the sole basis of specific toxicity of the different agents towards the four different groups of fungi, but rather through the selective action of the agents on the struggle for dominance on the agar medium between the different fungi in the straw. Calcium hypochlorite appears to be a mild sterilizing agent, and the fungus flora of *Fusarium culmorum*, *Trichoderma* spp. and *Mucor* spp. developing from the straw in this treatment is characterized by speed and density of growth rather than by high resistance to fungicidal action. Mercuric chloride, on the other hand, appears to be a particularly severe sterilizing agent and greatly simplifies the flora, which is reduced chiefly to *Penicillium* spp., characterized by relatively slow growth but high resistance to the fungicide. Silver nitrate appears to occupy a position intermediate in this respect between calcium hypochlorite and mercuric chloride.

One feature of the results calls for comment. The proportion of *Fusarium culmorum* to *Penicillium* spp. is much lower in the 2 min. treatment with mercuric chloride than in Exps. 1, 2 and 3 when using this surface-sterilizing agent. Times of sterilization in the preceding experiments were, however, shorter, being 1-1½ min. in Exps. 1 and 2 and a standardized 1½ min. in Exp. 3. An increase in the time to 2 min. in Exp. 4 has certainly much increased the *Penicillium* spp. at the expense of *Fusarium culmorum*. It seems likely, therefore, that part of the unexplained variation in the results of Exp. 1 could have been eliminated by more careful standardization of the time of immersion in the mercuric chloride.

Exp. 5. This experiment was planned to include a study of fungal colonization not only of sterile straws, but also of straws inoculated with, and completely permeated by, pure cultures of *Fusarium culmorum* and *Penicillium* sp., when subsequently buried in the soil.

The inoculated straws were prepared by a modification of Garrett's (1938) method. The *Fusarium culmorum* isolate was obtained from Stackyard soil during the course of Exp. 1; the *Penicillium* sp. from Great Harpenden soil, the isolate being taken from the series treated for 4 min. with mercuric chloride in Exp. 4.

The soil was taken from an outside stack of Great Harpenden soil, collected from the field in March 1940 and employed in Exps. 3 and 4, and subsequently in this experiment some 2 months later. The usual twenty-five straws were buried in each pot; four pots, comprising 100 straws, were taken from each series at each sampling, at 14, 28 and 56 days, respectively.

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The three surface-sterilizing treatments used in Exp. 4 were employed with one additional treatment, viz.

Sterile water. Washing in six changes of sterile tap water.

Calcium hypochlorite, treatment for 10 min.

Silver nitrate, treatment for 2 min.

Mercuric chloride, treatment for 2 min.

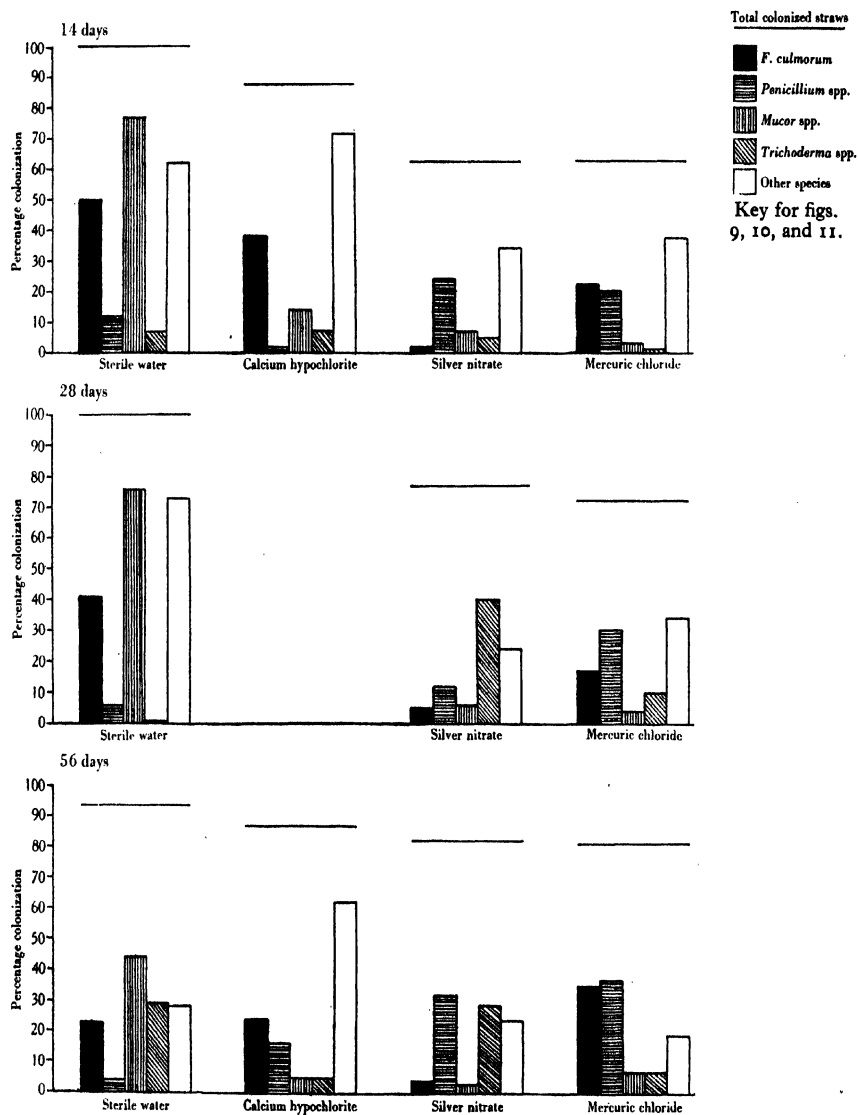


Fig. 9. Development of fungi from originally sterile straws buried in soil of Exp. 5, after treatment with different sterilizing agents.

Unfortunately both the originally sterile, and the *Penicillium*-inoculated series of straws treated with calcium hypochlorite at the 28-day sampling were spoilt through an accident in handling, and results for these two series are consequently lacking. The results of this experiment are given in Figs. 9-11.

Originally sterile straws (Fig. 9). The percentage colonization of straws by fungi was 100 in the 14- and 28-day samplings, and 93 in the 56-day sampling of the sterile water series, was somewhat reduced in the calcium hypochlorite series and still further reduced in the silver nitrate and mercuric chloride series.

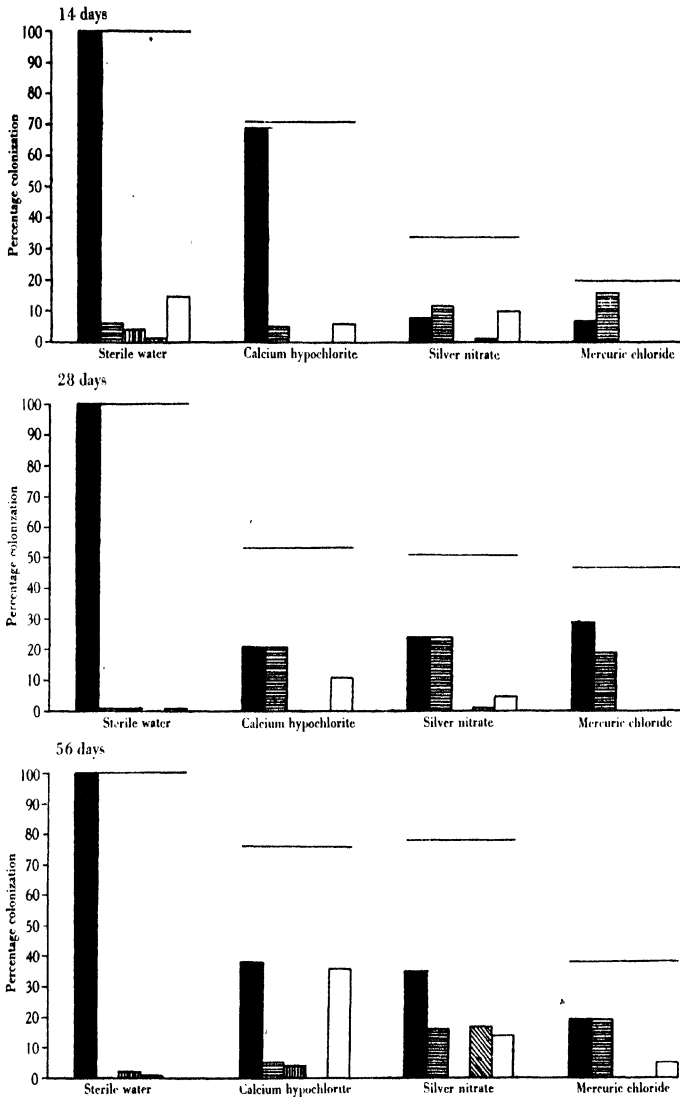


Fig. 10. Development of fungi from straws, originally inoculated with *Fusarium culmorum*, buried in the soil of Exp. 5, after treatment with different sterilizing agents.

Fusarium culmorum was subdominant to *Mucor* spp. in the 14- and 28-day samplings of the sterile water series and surpassed both by *Mucor* spp. and by *Trichoderma* spp. in the 56-day sampling. As in Exp. 4 it was dominant in the calcium hypochlorite series. In the silver nitrate series it was the lowest of all four groups of fungi in the 14- and 28-day samplings

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and lowest but one in the 56-day sampling. Taking the three samplings of the mercuric chloride series together, *Fusarium culmorum* might be classed as co-dominant with *Penicillium* spp. in this series.

Penicillium spp. were lower in the sterile water series than in the calcium hypochlorite

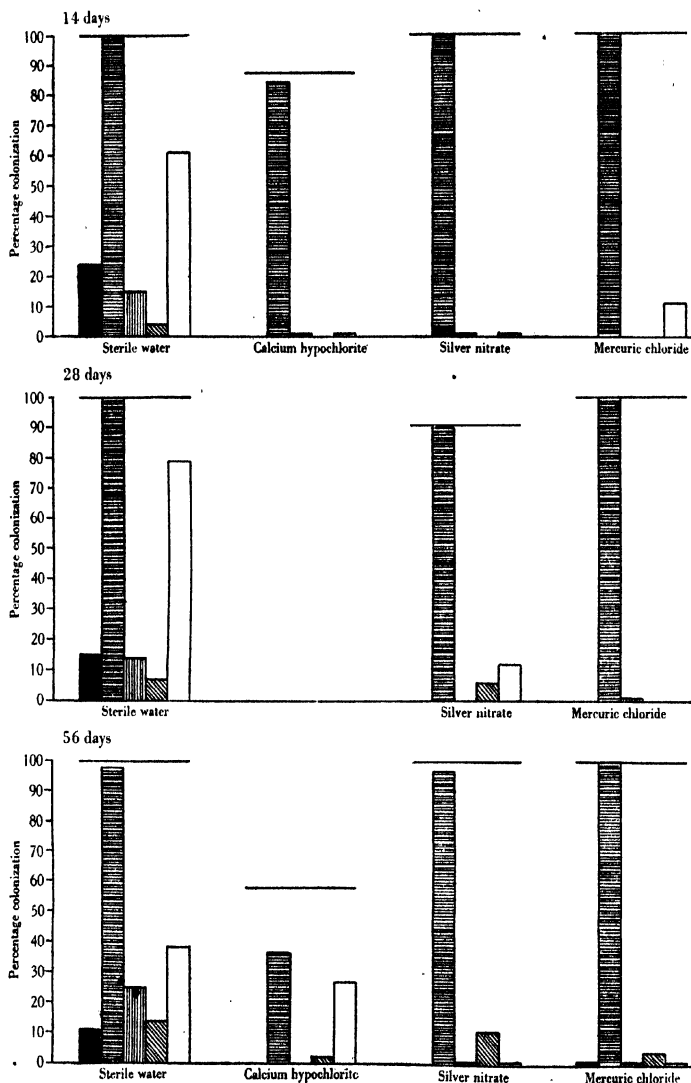


Fig. 11. Development of fungi from straws, originally inoculated with *Penicillium* sp., buried in the soil of Exp. 5, after treatment with different sterilizing agents.

series, and under both series were poorly represented. The poor development of *Penicillium* spp. in the sterile water series supports the suggestion advanced above that their sparse development in the calcium hypochlorite series in Exp. 4 was due less to any specific toxicity of the agent to *Penicillium* spp. than to the competition of more vigorously growing

species, which calcium hypochlorite had failed to suppress. In the three samplings of the silver nitrate series, *Penicillium* spp. were first dominant, then subdominant to *Trichoderma* spp. and finally co-dominant with that group. After the mercuric chloride treatment, *Penicillium* spp. appeared co-dominant with *Fusarium culmorum*.

Mucor spp. were dominant in the sterile water series, were displaced by *Fusarium culmorum* and later also by *Penicillium* spp. in the calcium hypochlorite series, and were relatively unimportant in the silver nitrate and mercuric chloride series.

Trichoderma spp. remained at a relatively low level in all series except in the last sampling of the sterile water series, when they were all subdominant to *Mucor* spp., and in the mid and last samplings of the silver nitrate series, when they were absolutely dominant and then co-dominant with *Penicillium* spp., respectively. As in Exp. 4, *Trichoderma* spp. appeared to be peculiarly tolerant of silver nitrate.

Straws originally inoculated with Fusarium culmorum (Fig. 10). The 4 weeks' incubation in the pure culture flasks at 25° C. after inoculation with *F. culmorum* and before burial in the soil seemed to have a remarkable effect upon the percentage colonization of the straws by fungi. Whilst this was 100 % (chiefly *F. culmorum*) in the series treated with sterile water, it was reduced to the 50–75 % level by calcium hypochlorite and still further by the silver nitrate and mercuric chloride treatments. In the two latter treatments percentage colonization increased in the mid and last samplings, especially in the case of the silver nitrate series. The effect of the sterilizing agents on percentage colonization may be partially explained by supposing that other fungi, more resistant to the sterilizing agents, had not established themselves sufficiently in the straws to take the place of the killed *F. culmorum* on the isolation plates. This suggestion is supported by the increase in the percentage colonization of the straws at the two latter samplings in the silver nitrate and mercuric chloride series. At the same time this increase is made up as much by an increase in the number of *F. culmorum* colonies as by an increase in the number of *Penicillium* spp. supposedly more resistant to the fungicides. The severe depression in fungal colonization can, therefore, be but partially explained by this suggestion.

Straws originally inoculated with Penicillium sp. (Fig. 11). Colonization of the straws was 100 % throughout in the sterile water series and in the mercuric chloride series; in the three samplings of the silver nitrate series it was 100, 90 and 97 % respectively. The percentage occurrence of *Penicillium* spp. also remained at almost 100 throughout these three series, although a number of the straws yielded other colonies as well, particularly in the sterile water series. An interesting feature of the *Penicillium* inoculated straws was the marked depression both in percentage straws colonized by fungi, and in the percentage colonized by *Penicillium* in the calcium hypochlorite treatment at the 56-day sampling. A smaller depression can be observed in the 14-day sampling.

Summarizing the results of Exps. 4 and 5, upon the action of different surface-sterilizing agents upon the straws originally sterile when buried, the numerically largest, and also the most varied fungus floras were observed to grow out from the straws washed in sterile water alone. *Mucor* spp. were dominant, with *Fusarium culmorum* tending to be subdominant. Calcium hypochlorite appeared to be a mild sterilizing agent which resulted in a comparatively large and varied fungus flora growing out from the straws, with *F. culmorum* tending to be dominant. This agent appeared to have a specifically toxic effect upon *Penicillium* spp., and the latter were probably further suppressed by the more vigorous

growth of other species relatively tolerant of calcium hypochlorite. Silver nitrate reduced and simplified the fungus flora derived from the straws; *Penicillium* spp. tended to be dominant, but were sometimes replaced by *Trichoderma* spp., which appeared specifically tolerant of this sterilizing agent. Mercuric chloride, especially in the longer treatment, also tended to reduce and simplify the fungus flora obtained from the straws; with shorter times of treatment *Fusarium culmorum* might be dominant, but as length of treatment increased *Penicillium* spp. increased until they might become the sole fungus obtained from the straws. *Penicillium* spp. therefore appeared to be particularly tolerant of mercuric chloride.

DISCUSSION

In the experiments described, the importance of *Fusarium culmorum* as a colonizer of buried wheat straw has been demonstrated in a number of cultivated soils, at all seasons of the year. Sadasivan's (1939) hypothesis that the relative colonizing activity of *F. culmorum* and other soil fungi might fluctuate according to the season was not confirmed by the experiments; nevertheless, it must not be concluded that no such fluctuation occurs. Subsequent experiments have demonstrated that the growth of fungus colonies from the straws, in addition to being affected by the period of incubation of the straws in the soil, is dependent on the surface-sterilizing agent used and the time of immersion in that agent; thus, had the period of immersion been more strictly controlled in Exp. 1, it is possible that some of the unexplained variations in colonization would have been eliminated. Sadasivan's conclusions as to the succession of fungi on buried wheat straw have been confirmed only in part.

The experiments have demonstrated the equal importance of the group of *Penicillium* spp. as colonizers of buried straw, and have indicated an inverse relationship between the occurrence of *Penicillium* spp. and *Fusarium culmorum* on the plates. The struggle for dominance between *Penicillium* spp. and *Fusarium culmorum* in the buried wheat straw is evidently affected by the process of surface sterilization, which may actually reverse the final issue of the struggle when the straws are plated out (Exps. 4 and 5).

The saprophytic isolates of *Fusarium culmorum* from buried wheat straw have proved to be just as pathogenic to wheat seedlings as those isolated from diseased plants; straws invaded by *F. culmorum* as a saprophyte may therefore form potential centres for infection of the underground parts of cereal plants coming into contact with them, as well as a means of perpetuation of the organism in the soil.

In this connexion particular interest attaches to the observations of Russell (1934) in Saskatchewan and of Sanford (1939) in Alberta that whereas the incidence of take-all (*Ophiobolus graminis*) of wheat was markedly reduced by crop rotation, that of common rot (*Fusarium culmorum* and *Helminthosporium sativum*) was scarcely affected. Tyner (1940) has investigated the effect of adding wheat, oat and barley straw, respectively, upon the development of disease in successive crops of wheat seedlings grown in pots originally inoculated at seed-level with cultures of *Fusarium culmorum*, *Helminthosporium sativum* and *Ophiobolus graminis*, respectively. Whilst wheat straw tended to be the most, and oat straw the least, favourable to the development of disease in the seedlings, the effects were not consistent in successive plantings, nor could any relation be established between the development of disease and the amount of straw added.

SUMMARY

A study has been made by Sadasivan's (1939) technique of the colonization by fungi of wheat straw buried in the soil. One-inch lengths of sterilized straw were buried in the experimental soils in $3\frac{1}{2}$ in. flower pots; after incubation at laboratory temperature ($16-20^{\circ}\text{C}.$) for the required period the straws were washed out of the soil, surface sterilized by mercuric chloride or other sterilizing agent and plated out on acidified potato-dextrose agar ($\text{pH } 5.0$).

Fusarium culmorum and *Penicillium* spp. were numerically the most important organisms developing from the straws on the plates, at least during the first 5 months of incubation in the soil. Both groups of organisms, together with others, appeared generally to be present in the decomposing straw, but the method of surface sterilization employed apparently decided which organism produced a colony on the isolation plate.

Fusarium culmorum, a fungus of a vigorous and rapid habit of growth, showed low resistance to the action of the more severe sterilizing agents, such as mercuric chloride and silver nitrate, but developed better after surface sterilization of the straws with calcium hypochlorite, a mild sterilizing agent, and best of all after a mere washing in sterile water. *Penicillium* spp. were apparently crowded out by the more vigorous growth of *Fusarium culmorum* after these mild treatments of the straws; on the other hand, they were very tolerant of the more severe surface sterilizing agents, mercuric chloride and silver nitrate, and after the longer period of treatment were often the only organisms developing on the plates.

The pathogenicity to wheat seedlings of the isolates of *F. culmorum* obtained from decomposing wheat straw was shown to be comparable with that of isolates of the same fungus secured from diseased cereal plants.

I am indebted to Mr S. D. Garrett for suggesting this problem, and for his interest and helpful criticism throughout the course of the investigations. Thanks are also due to Miss M. D. Glynne for assistance in the identification of some of the genera mentioned. A portion of the work was carried out during my tenure of an Agricultural Research Scholarship.

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A QUANTITATIVE STUDY OF THE INTERACTION OF VIRUSES IN PLANTS

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(With Plate 16 and 1 Text-figure)

ATTEMPTS to infect plants with mixtures of virus strains led to the discovery that plants infected with one strain acquire an immunity, or resistance, to infection with other strains of that virus, although still remaining susceptible to unrelated viruses (Thung, 1931; Salaman, 1933, 1938; Kunkel, 1934; Caldwell, 1935). The mechanism underlying this acquired immunity is unknown, but it is of the non-sterile type, the immunity existing only so long as a plant contains one strain in an active form. Most workers have merely shown that plants systemically infected with avirulent strains fail to develop any further symptoms when reinoculated with virulent strains. In the work described in this paper an attempt has been made to apply quantitative methods to the problem, the actual extent of resistance acquired by infection with one strain being measured by counts of local lesions and not by eye observation of the severity of systemic symptoms.

METHODS

So that some idea of the general application of the results could be obtained, the experiments were done with both potato virus "X" and tobacco mosaic virus, two unrelated viruses with widely different properties. Two strains of each virus were used, *S* and *G* of potato virus "X" (Salaman, 1938) and tobacco mosaic virus and tomato aucuba mosaic virus. "X^s" causes necrotic local lesions and necrotic systemic symptoms in both tobacco (var. White Burley) and *Nicotiana glutinosa*, whereas "Xⁿ" causes only a faint systemic mottle in these plants. Common tobacco mosaic virus causes necrotic local lesions in *N. glutinosa* and systemic mottling symptoms only in *N. sylvestris* and tobacco, whereas aucuba mosaic virus causes necrotic local lesions in all three; aucuba mosaic virus usually becomes systemic in tobacco and rarely does so in *N. sylvestris*.

In this work the experimental designs usually employed for lesion counts, such as the half-leaf method and the Latin square, could not be used, for one-half of the inoculations with either "X^s" or aucuba mosaic virus had to be made to healthy plants and the other to plants already infected. In each experiment, therefore, the treatments were all replicated at least ten times, to reduce differences arising from individual plant variations. As it was also frequently necessary to use leaves of different sizes and ages, the usual method of recording lesions as number per leaf had to be abandoned. Instead they were recorded as the numbers per 100 sq. cm. of inoculated leaf. To obtain the area of the leaves their lengths and breadths were measured and the products of these multiplied by a factor which was found to be constant for each species. The areas of sixty leaves each of tobacco, *N. sylvestris*, and *N. glutinosa* were found by tracing on squared paper, and their lengths and breadths at the widest points measured. In spite of the difference in sizes and ages of the leaves measured, the ratio length times breadth to area was practically constant for each species. That for tobacco varied from 0.67 to 0.73 (average 0.7), for *N. sylvestris* from 0.72 to 0.76 (average 0.74), and for *N. glutinosa* from 0.87 to 0.89 (average 0.88).

Inoculations were made by rubbing whole leaf surfaces as evenly as possible with the forefinger dipped in the experimental fluids. Infective preparations of three kinds were used: (1) crude saps, (2) clarified saps prepared by heating to 60° C., and centrifuging for 10 min. at 3000 r.p.m., and (3) purified virus preparations made by the methods described by Bawden & Pirie (1937).

EXPERIMENTAL

In preliminary experiments the whole leaf surfaces of plants were rubbed with either water, or crude sap from plants infected with tobacco mosaic virus, potato virus "Y", or potato virus "X^G". At daily intervals these leaves were rubbed over their whole surfaces with crude aucuba mosaic sap diluted 1/100 with water. The average number of lesions per 100 sq. cm. produced on these plants is given in Table 1. It will be seen that the only plants to become resistant to aucuba mosaic virus were those that had previously been inoculated with tobacco mosaic virus. These show a steady decrease in susceptibility to aucuba mosaic virus; with increasing time they were infected with tobacco mosaic virus up to 6 days when they were quite immune. The aucuba mosaic lesions on plants infected with "X^G" or virus "Y" were similar to those on the healthy controls, large circular necrotic spots, whereas, on those infected with tobacco mosaic virus, in addition to being fewer, the lesions were smaller and had much more uneven edges. In similar experiments in which rubbed plants were reinoculated at intervals with "X^S", the only plants to show a reduction in susceptibility were those previously rubbed with "X^G". These tests sufficed to show that the acquired immunity is specific, being restricted to strains of one virus.

Although the presence of an unrelated virus in a plant had no effect in the number or type of lesion produced by aucuba mosaic virus or "X^S", unrelated viruses may have interactions inside a plant; e.g. tobacco mosaic virus and potato virus "X" together cause acute necrotic diseases in tobacco and tomato that neither can cause alone. Similarly, in these local lesion tests, it has been found that plants infected with tobacco mosaic virus show necrotic, spot-like local lesions when reinoculated with "X^G" and vice versa, although neither virus alone produces visible local lesions on this plant.

TABLE 1. *Effect of previous inoculation of plants on number of local lesions produced by aucuba mosaic virus*

Days between inoculations	No. of lesions per 100 sq.cm. on plants previously rubbed with			
	Water	Virus "Y"	Virus "X ^G "	Tobacco mosaic
1	675	762	639	220
2	715	675	577	185
3	765	650	578	80
4	718	602	675	70
5	635	627	716	10
6	618	687	710	0
7	575	615	680	0
8	645	610	600	0
9	712	627	575	0
10	676	595	585	0

These results could be explained on two hypotheses: (1) that related viruses have a mutually destructive effect or (2) that in the plant there is an intense competition between them. To test the first possibility a number of *in vitro* tests were made. Crude "X^S" sap was mixed in different proportions with sap from plants infected with "X^G" and with sap from healthy tobacco plants and from those infected with various viruses, and these mixtures were tested for their infectivity. Tests were also made with aucuba mosaic virus diluted similarly. The results of one such experiment are given in Table 2.

TABLE 2. *Effect on infectivity of "X^S" and aucuba mosaic virus of diluting in different fluids*

Preparation diluted	Diluent	No. of lesions at dilution of				
		1/20	1/100	1/500	1/2500	1/12,500
Crude "X ^S "	Tobacco sap	196	54	20	4	1
	Crude virus "Y" sap	180	85	37	8	2
	Crude mosaic virus	207	64	34	6	1
	Crude "X ^U " sap	30	9	0	0	0
	Water	361	183	62	7	4
Aucuba mosaic	Tobacco sap	134	86	32	23	8
	Crude "X ^U " sap	131	78	21	11	2
	Crude mosaic virus	43	15	4	0	0
	Water	383	346	180	58	18

Several similar experiments have been done with the same results, saps from healthy and infected plants inhibiting the infectivity of both "X^S" and aucuba mosaic virus. The sap from plants infected with unrelated viruses has no significantly different effect from healthy plant sap, but that containing related viruses has a much greater inhibitory action. In addition to those given in Table 2, "X^S" and aucuba mosaic virus were mixed with sap expressed from plants infected with the following viruses: *Hyoscyamus* 3, tobacco etch, tobacco ringspot, and cucumber virus 1. All had the same inhibitory effect as healthy plant sap. On the other hand when "X^S" was mixed with sap from plants infected with "X^U", greater inhibition was obtained, comparable with that produced by "X^G". The materials in healthy and infective saps responsible for the non-specific reduction in infectivity are not removed by centrifugation at 3000 r.p.m. after heating to 60° C., for in experiments comparing the inhibitory effects of crude and clarified saps no significant differences were obtained. The greater specific reduction in infectivity caused by diluting with sap containing related viruses seems to be due to the presence of the viruses themselves and not to any other specific product of virus activity, for a similar reduction in the infectivity of purified aucuba mosaic virus occurs when it is diluted with purified tobacco mosaic virus free from all detectable impurities. Similarly, when aucuba mosaic virus is mixed with crude boiled sap of tobacco mosaic virus, the lesion production of aucuba mosaic is almost identical with that produced when mixed with healthy sap of tobaccos (Pl. 16, fig. 1).

At first sight the results of these dilution experiments suggest that related viruses may have a mutually inactivating effect *in vitro*. This is especially so as the reduction in the number of lesions produced by crude aucuba mosaic sap diluted 1/100 with tobacco mosaic sap is greater than that when plants are first inoculated with the tobacco mosaic virus and then reinoculated with aucuba mosaic virus diluted 1/100 with water. However, the results of other experiments show that any interactions between related viruses occur within the plant and not *in vitro*. One of the two effects *in vitro* might cause loss of infectivity; tobacco mosaic might actually destroy aucuba mosaic virus or it might act merely as an inhibitor of infectivity such as trypsin and some other proteins do. If the first were true incubating the mixtures would be expected to give increased reduction of lesions, for such inactivation would not be expected to occur immediately. However, no significant differences were found between the number of aucuba lesions when the mixtures were rubbed on to tobacco or *N. sylvestris* immediately and when they were incubated for some hours. If tobacco mosaic virus merely acts as an inhibitor, its effects would be expected to be determined by

the total amount of protein present, whereas it is not. The infectivity of tobacco mosaic virus can be destroyed with formaldehyde (Stanley, 1934) without denaturing the protein or altering most of its physical and chemical properties. Mixtures of purified aucuba mosaic virus were made with the same amount of purified tobacco mosaic virus which in some was fully active and in others inactivated by varying amounts with formaldehyde. The reduction in lesions when these mixtures were inoculated to tobacco and *N. sylvestris* was always proportional to the amount of infective tobacco mosaic virus. If either of these *in vitro* reactions occurred loss of infectivity would be expected on every host plant, but this is not so. The reduction only happens on host plants in which aucuba mosaic produces local lesions and tobacco mosaic virus becomes systemic. When dilute mixtures of the two viruses were inoculated to *N. glutinosa*, in which both give only local lesions, there is no evidence of inhibition of the local lesion production of either of the viruses, for the number of lesions produced are what would be expected from the total amount of virus present in the mixtures.

The results so far examined show that virus strains producing systemic symptoms reduce the number of lesions caused by other strains when mixed *in vitro*. This suggests that any interaction occurs within the plant. Further experiments with tobacco mosaic, aucuba mosaic, potato virus "X^S" and "X^G" *in vivo* have lent support to this view and suggest that in the plant tissues there is an intense competition between related viruses, and that the resistance acquired to one depends on the amount of the other already present. Experiments were performed where different parts of leaves of tobacco were inoculated with tobacco mosaic and "X^G" viruses and such areas reinoculated with aucuba mosaic and "X^S" viruses respectively. In one of these experiments *tips* of leaves were inoculated with either "X^G" sap or tobacco mosaic sap and then reinoculated with "X^S" or aucuba mosaic virus at daily intervals. The "X^G" areas were quite immune to "X^S" after 12 days and the tobacco mosaic areas immune to aucuba mosaic virus in 4 days. The *bases* of such leaves showed an incomplete inhibition even after 20 and 24 days. When *bases* of leaves were similarly inoculated, essentially the same type of inhibition was obtained in the "X^G" and tobacco mosaic inoculated areas, but the *tips* of such leaves acquired resistance to "X^S" and aucuba mosaic much more slowly and incompletely. The results of this experiment are given in Table 3.

In addition to the leaves reinoculated with aucuba mosaic virus some of the *N. sylvestris* plants inoculated in *tips* and *bases* with tobacco mosaic virus were sampled at different periods of incubation by cross inoculation on to *N. glutinosa* for their virus content. Inoculated areas, whether *tips* or *bases*, soon contained a high concentration of the virus, whereas virus entered and increased slowly in uninoculated areas. When *tips* of leaves were initially inoculated, the virus moved down to the *bases* and reached a high concentration whereas, with the leaves where *bases* were inoculated, the *tips* rarely obtained such a high concentration. This once again corroborates Samuel's (1934) suggestion that the movement of viruses is related to the translocation stream in the plant and that therefore movement from *tips* to *bases* is more efficient than *bases* to *tips*. Although this experiment could not be repeated for "X^G" virus, which does not produce visible local lesions, it probably moves and multiplies in a similar way though at a lower level than tobacco mosaic virus.

Thus, it appears from the above experiments and the data presented in Table 3 that "X^G" and tobacco mosaic virus bring about rapid inhibition to "X^S" and aucuba mosaic virus in the initially inoculated areas, but further inhibition in other parts of the plant depends on the efficient spread of "X^G" and tobacco mosaic virus from the inoculated areas

TABLE 3. Comparison between "X^S" and aucuba mosaic lesions produced in tips and bases of leaves inoculated with "X^G" and tobacco mosaic viruses in *N. Tabacum* and *N. sylvestris* respectively

Period of incubation days	No. of lesions per 100 sq. cm. leaf area							
	Tips alone inoculated with				Bases alone inoculated with			
	"X ^G "		T.M.V.		"X ^G "		T.M.V.	
	Tips	Bases	Tips	Bases	Bases	Tips	Bases	Tips
1	723	829	163	405	380	623	101	650
2	583	540	76	371	451	664	118	489
3	331	612	32	226	253	729	34	260
4	406	568	8	402	190	603	14	222
5	18	411	0	385	26	977	0	231
6	8	462	0	365	4	656	0	208
8	16	145	0	112	9	600	0	138
10	8	87	0	317	3	262	0	109
12	5	110	3	48	12	212	0	53
14	0	195	6	75	0	177	5	26
16	0	155	8	13	0	215	9	49
20	0	18	13	12	0	265	8	50
22	—	—	3	8	—	—	10	38
24	—	—	3	9	—	—	9	42

into the adjoining leaf tissues, and their multiplication therein. In other words the higher the concentration of the two strains the greater the inhibition to "X^S" and aucuba mosaic virus. This process of natural movement within the host, however, does not seem to bring about as complete or as rapid an inhibition as direct inoculation of areas. The results suggest that the direction of movement of viruses is dependent on the place of inoculation, for it has been shown that movement from *tips* to *bases* of leaves is comparatively more efficient than from *bases* to *tips* as also evidenced by inhibition tests of "X^S" and aucuba mosaic viruses. Since all these observations were made on parts of leaves of one age, further experiments were conducted with tobacco mosaic and aucuba mosaic viruses on young, medium and old leaves of *N. sylvestris*. Inoculations were made as follows: three sets of plants were inoculated with tobacco mosaic virus on one leaf each of three ages, young, medium and old. These plants were reinoculated at 2-day intervals up to 20 days with aucuba mosaic virus on the initially inoculated leaves as well as on the other uninoculated leaves of three ages. The results, Table 4, show that tobacco mosaic inoculated areas in all three ages completely resist aucuba mosaic lesions at the end of 12 days. In contrast young leaves that were not initially inoculated with tobacco mosaic virus although resistant are not immune even after 20 days, though tobacco mosaic systemic symptoms appear after 10 days' incubation. Such incomplete inhibition, however, is greater in uninoculated medium-aged leaves and more so in old leaves.

In experiments where three ages of leaves from *N. sylvestris* plants were infected with tobacco mosaic virus and were tested on to *N. glutinosa* at different periods of incubation it was found that whatever the age of the leaf receiving the initial inoculation the young leaves always have a higher concentration of the virus than medium and old leaves that become systemically infected. This result contrasts with that obtained when leaves are inoculated and not infected by systemic spread, for then the virus multiplies more rapidly and reaches a higher concentration in medium and old leaves than in young. Indeed, the concentration

of virus in directly inoculated old leaves is almost twice that in the young ones. Correspondingly, the data in Table 4 show that medium and old leaves inoculated with tobacco mosaic virus show a large reduction in the aucuba mosaic lesions in 2 days, whereas similarly treated young leaves take as long as 4 days to bring about the same reduction. One such comparison between the concentration of tobacco mosaic virus in inoculated leaves of *N. sylvestris*, as shown by tests on *N. glutinosa*, and their susceptibility to aucuba mosaic virus is presented in Text-fig. 1. It will be seen that as the concentration of tobacco mosaic virus increases with increased incubation, the number of aucuba mosaic lesions steadily decreases, i.e. the rise of concentration of tobacco mosaic brings about a corresponding rise in the degree of resistance to aucuba mosaic virus.

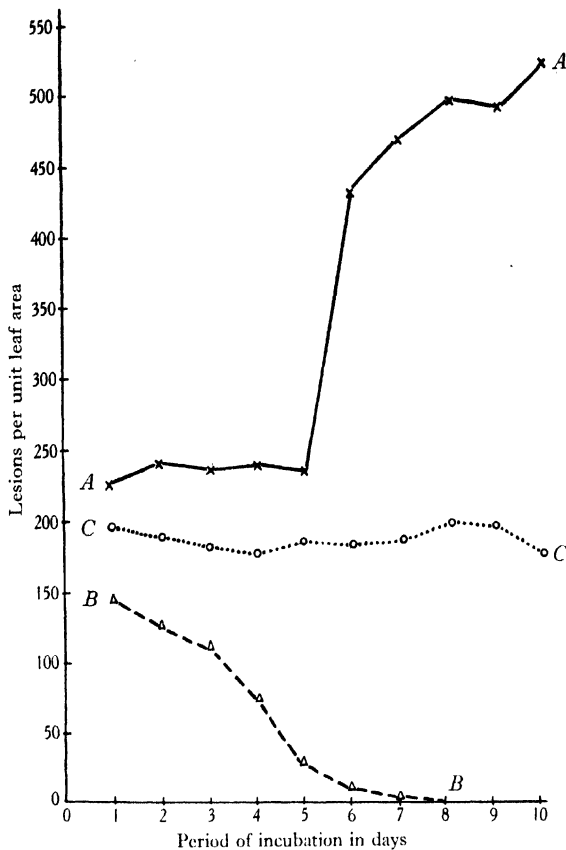
TABLE 4. *Comparison of aucuba mosaic lesions between leaves of three ages where tobacco mosaic virus has been inoculated and also on leaves of the three ages when tobacco mosaic systemic symptoms have appeared*

Period of incubation days	Young leaves directly inoculated with T.M.V.	Young leaves showing T.M.V. systemic symptoms	Medium leaves directly inoculated with T.M.V.	Medium leaves showing T.M.V. systemic symptoms	Old leaves directly inoculated with T.M.V.	Old leaves showing T.M.V. systemic symptoms
2	227	196	350	826	346	1830
4	205	145	337	1166	272	1330
6	187	133	167	979	296	1450
8	100	75	134	484	127	1418
10	23	67	113	584	25	1612
12	8	63	50	557	0	1716
14	0	50	0	603	0	961
16	0	69	0	613	0	824
18	0	79	0	392	0	875
20	0	15	0	253	0	818

Although old and medium leaves, when directly inoculated with tobacco mosaic virus, become resistant to aucuba mosaic virus more rapidly than young ones, the reverse is true when infection occurs as a result of systemic spreading of tobacco mosaic virus. This again is correlated with virus content, for although tobacco mosaic virus multiplies more rapidly in old and medium leaves when they are directly inoculated, when systemic infection occurs multiplication is more rapid and the highest virus content is found in young leaves.

As these observations suggest that the inhibition of aucuba mosaic virus lesions is directly proportional to the concentration of the tobacco mosaic virus in the leaf tissue, regardless of the age of the leaf, it seemed that any method of increasing virus concentration should increase acquired immunity. It is known that carborundum powder incorporated in the inoculum increases the number of local lesions. This increase is believed to result from an abrasive action increasing the number of entry points. A comparison was, therefore, made between sets of *N. Tabacum* and *N. sylvestris* leaves inoculated with crude tobacco mosaic virus sap and another sap to which was added carborundum powder.

These inoculations were made in the centres of laminae and aucuba mosaic virus was reinoculated all over the surface of the leaves at different periods of incubation. The results presented in Table 5 show that a more efficient and earlier inhibition of aucuba mosaic virus could be obtained when carborundum is in the tobacco mosaic virus inoculum than when it is not (Pl. 16, fig. 2).



Text-fig. 1. Correlation between virus content and acquired immunity: curve A, tobacco mosaic virus content of *N. sylvestris* as shown by subinoculating to *N. glutinosa*; curve B, aucuba mosaic lesions on tobacco mosaic infected *N. sylvestris*; curve C, aucuba mosaic lesions on water-rubbed control of *N. sylvestris*.

TABLE 5. Comparison between aucuba mosaic lesions produced in tobacco mosaic inoculated laminae with and without carborundum powder

Period of incubation of T.M.V. days	No. of lesions per 100 sq. cm. leaf area											
	T.M.V. + carborundum						T.M.V. alone					
	<i>N. Tabacum</i>			<i>N. sylvestris</i>			<i>N. Tabacum</i>			<i>N. sylvestris</i>		
	Tips	Centres*	Bases	Tips	Centres*	Bases	Tips	Centres*	Bases	Tips	Centres*	Bases
1	1590	321	1285	432	233	200	1470	310	1060	462	296	368
2	1060	60	513	626	283	303	1154	100	977	687	205	400
4	878	0	805	194	15	312	975	35	1052	436	36	457
6	979	0	741	172	16	141	867	8	847	486	32	367
8	825	0	155	160	0	23	713	0	390	240	13	65
10	430	0	15	175	0	65	452	0	140	218	0	16
15	120	0	4	20	0	0	148	0	12	34	0	10

* Areas inoculated with tobacco mosaic virus.

The response to the inhibition of aucuba mosaic lesions in the tobacco mosaic inoculated areas in both types of inoculation is quicker and more efficient in tobaccos than in *N. sylvestris*. Since aucuba mosaic virus produces more lesions in tobaccos than in *N. sylvestris* it could, perhaps, be assumed that *N. Tabacum* is more susceptible than *N. sylvestris* both to aucuba mosaic as well as tobacco mosaic viruses, although tobacco mosaic virus does not produce visible necrotic lesions in either of the two hosts. On this assumption the number of tobacco mosaic virus infective units entering tobacco leaves would be greater than those entering *N. sylvestris* leaves. Thus it seems that *N. Tabacum* acquires resistance to aucuba mosaic virus when vaccinated with tobacco mosaic virus more rapidly than *N. sylvestris* because it is more susceptible to both the viruses.

The only evidence suggesting that the immunity is not merely a result of competition between related virus strains in the plant, is that presented by experiments on mixing viruses *in vitro*. Table 1 shows that mixing of either aucuba mosaic virus with tobacco mosaic or " X^S " with " X^U " viruses produces a specific reduction in the lesions of the former viruses approximately 10 times that produced when mixed with healthy saps. Such a great reduction, however, does not happen when plants inoculated with tobacco mosaic virus or " X^G " are reinoculated with aucuba mosaic virus or " X^S " after 1 day. This apparent anomaly may result from a second type of competition that would occur only when the two strains were inoculated together, i.e. a competition for entry points. In experiments where two viruses were mixed, the concentration of tobacco mosaic virus and " X^U " used for the *in vitro* mixture has always been greater than either aucuba mosaic or " X^S " viruses. A competition for the fixed number of entry points follows, and since the higher number of tobacco mosaic virus and " X^G " infection units utilize the majority of the entry points, only a few are made available for aucuba mosaic and " X^S " virus units. It is highly probable that when tobacco mosaic virus is incubated in the leaves for 1 day, one set of entry points is occupied by the virus, and when such leaves are reinoculated with aucuba mosaic, a fresh number of entry points is made available in the second abrasive process of reinoculation.

Experimental evidence so far examined shows that there is a competition between related strains of viruses. Whether this competition is for a limited amount of material from which viruses reproduce or for the limited parts of a cell wherein the multiplication occurs there is no evidence to suggest. However, summing up the data, *acquired immunity seems to depend on the presence of virus units in a fully active state in cell tissues where they prevent the multiplication of another strain of that virus. This process is highly specific, being confined to the related groups only, and the efficiency merely rests on the concentration and uniform distribution of the virus units, either under natural movement within the plants themselves, or, by direct introduction of active virus units into the cell tissues by mechanical rubbing. The degree of immunity is directly proportional to the number of active units of the protecting strain entering the host cells.*

SUMMARY

When the saps of healthy plants are mixed with potato virus " X^S " or aucuba mosaic virus *in vitro* there is an inhibition of the lesion production on *N. Tabacum* and *N. sylvestris* leaves. Saps containing unrelated viruses also reduce the infectivity to the same extent as healthy saps. However, saps containing strains of related virus, have a greater and specific inhibitory action. Experiments were performed to show that this specific reduction produced



Fig. 1.

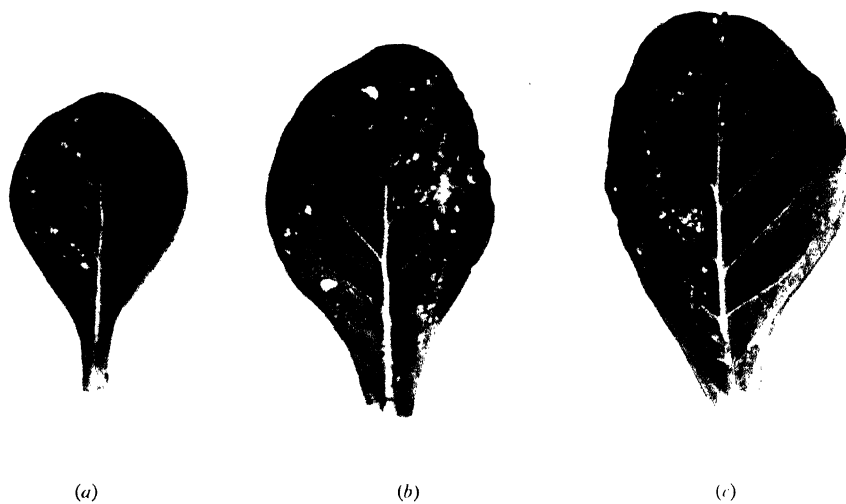


Fig. 2.

by related strains of viruses is due to the viruses themselves and not to other metabolic products present in the saps.

Further study by inoculating related strains of viruses *in vivo* has shown that the local lesions of one strain are inhibited by another when the latter systemically infects hosts, and that the efficiency depends on the concentration of the systemically infecting viruses in such hosts. The degree of inhibition of aucuba mosaic virus is directly proportional to the number of active units of tobacco mosaic virus present in the leaf tissues at the time of reinoculation.

I wish to express my indebtedness to Dr J. Henderson Smith and Mr F. C. Bawden for suggesting this problem. Their generous help and criticisms during the various stages of this piece of work are gratefully appreciated.

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EXPLANATION OF PLATE 16

- Fig. 1. Lesion on *N. Tabacum* leaves inoculated with aucuba mosaic virus sap mixed *in vitro* as follows: (a) with concentrated active tobacco mosaic sap; (b) with boiled tobacco mosaic sap; and (c) with healthy sap of tobacco.
- Fig. 2. *N. sylvestris* half leaves of three ages initially inoculated with tobacco mosaic virus, with and without carborundum powder. Lesions are of aucuba mosaic virus reinoculated after incubation of 1 day. Initial inoculations were: leaf (a) left, tobacco mosaic virus alone and right, tobacco mosaic virus + carborundum powder; leaf (b) left, tobacco mosaic virus alone and right, water-rubbed control; leaf (c) left, water + carborundum-rubbed control and right, tobacco mosaic virus + carborundum powder.

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150. THE INACTIVATION OF SOME PLANT VIRUSES BY UREA

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INTRODUCTION

OF the simple organic substances known to affect proteins in neutral solution, urea has been the most extensively studied. There is no obvious reason for this, for urea is neither a more powerful denaturing agent nor a better protein solvent than some of the others. The fact that it is a normal metabolic product is not really relevant, for most of the effects studied are observed only at 10 or more times the physiological concentration. Detailed studies on the action of urea have been made only in recent years, but some of its more striking effects were early noticed.

Buchner [1874; 1876] reviewed and extended earlier physiological studies on muscle stimulation, and noticed that within a few minutes of putting strips of muscle in concentrated urea solutions there was osmotic shrinking, followed by great swelling, though little of the material went into solution. Limbourg [1887] made similar experiments with frog nerves and seems to have been the first to discover that urea solutions could dissolve some otherwise insoluble proteins. He described the behaviour of such solutions towards commonly used protein precipitants and in 1889 found that solutions of fibrin in urea did not coagulate when heated. Loss of heat coagulation in 10% urea solutions was found with Bence-Jones protein by Magnus-Levy [1900] and with the commoner proteins by Ramsden [1902], who also described graphically the disintegration of a frog in saturated urea solution, and recommended the latter as a histological reagent. Braun [1933] and Stein & Miller [1938] adopted this recommendation for the histological recognition and preparation of elastin, which is one of the few proteins insoluble in concentrated urea solution. The solvent properties of urea have also been used in the preparation of other proteins, e.g. by Cook & Alsberg [1931] for glutenin, by Urban [1936] for a liver globulin mixture, by McKay & Lamson [1936] for pollen proteins and by Kondo *et al.* [1939] for soya bean proteins. Walker [1940] extracted antigens from *Salmonella aertrycke* with urea, but the method is not generally applicable, for Fuller [1938] and Miles & Pirie [1939] found it of no value with other bacteria.

Many workers have found that the presence of urea protects proteins from the precipitating effects of some reagents. Pauli & Rona [1902], Arnd & Hafner [1926], Jirgensons [1936] and Heim [1937] showed that more salt was necessary to get precipitates in solutions of gelatin, euglobulin, haemoglobin and fibrinogen if urea was present. Moser [1927] found serum proteins more difficult to precipitate by dilution or acidification in the presence of urea, and Ogiu & Pauli [1932] stabilized the complex of serum albumin and colloidal gold with urea and with glycine.

Detailed studies of the changes occurring when a protein is dissolved in strong urea solution, and the correlation of these with observations made in the presence of other organic substances, date from Spiro's [1900] work on their effect on coagulation by heat. Ramsden [1913], however, was the first to relate these changes with the group of protein-modifying processes known as denaturation. Although Ramsden's results were published in both English and German [Ramsden & Chavasse, 1913], they attracted little attention, and widespread interest was not aroused until Anson & Mirsky [1929] studied the denaturation of haemoglobin by urea and some other substances. Burk & Greenberg [1930], while continuing their earlier [1928] measurements of the osmotic pressure of proteins in 40% urea solution, described the denaturation of egg albumin and horse haemoglobin, and found that the osmotic pressure of haemoglobin denatured in this way corresponded to a molecular weight of 34,000, i.e. one-half of the original value.

Hopkins [1930] found that denaturation of egg albumin in the presence of urea proceeded more rapidly at 0° than at 37°, an interesting observation confirmed by Ramsden [1930]. Stanley & Lauffer [1939] found that the disintegration of tobacco mosaic virus also proceeded more rapidly at 0° than at 25°, but that it proceeded more rapidly at 40° than at 25°. Whether this increase in denaturation rate at low temperatures applies at all generally to proteins is uncertain, for Diebold & Jühling [1938] found that fibrinogen was rapidly denatured at 37°, whereas at 4° it was more stable in the presence of 23% urea than it was in water. However, the behaviour of fibrinogen seems to depend on its purity, as Diebold [1938] found that it was completely destroyed at 4° if urea was added to unfractionated plasma. Jühling & Wöhlisch [1938], who confirmed the difference between plasma and isolated fibrinogen, suggested that it may result from the activation of a fibrinolytic enzyme normally present in plasma in an inactive form.

1. *Evidence for the attachment of urea to proteins*

Observations similar to those of Buchner [1876] on the swelling of cornea in urea solutions have been made by Stoeltzner [1925] and Stoye [1925] on pieces of dura mater. Lloyd & Marriott [1933] measured the swelling of silk fibres in urea solution and found that there was a weakening of the lateral binding forces of the fibres. They suggested that urea was absorbed on the internal surfaces of the fibres, a conclusion agreeing well with the observation of Trogus & Hess [1933] that silk fibroin, after soaking in 50% urea, gave an X-ray pattern showing a regular arrangement of urea within the fibre. On the other hand, Astbury *et al.* [1935] do not mention such a pattern in washed films of denatured edestin or egg albumin prepared from urea solutions. Worschitz & Herman [1934] found that the micelle structure of muscle fibres disappeared in urea solutions and did not reappear when the urea was washed out. The phenomena of the swelling of crystalline or fibrous structures with urea and other substances were discussed by Katz [1932] and by Lloyd & Shore [1938]. Insoluble proteins that are not fibrous have been little studied, although Fischer & Sykes [1915] published some observations on the swelling of blocks of gelatin in solutions of urea too dilute to cause solution.

Attempts to use urea to measure the "bound water" in proteins have given further evidence that the first step in the action is the binding of urea to the protein. For example, Oda [1930] found that 5% of the water in serum was not available for dissolving glucose, whereas it was all available for the solution of urea. Similarly, Versmold [1931] measured the freezing points of solutions of

egg albumin containing either glucose, glycerol or urea and found that much more of the urea was bound to the protein. Finally, measurements of the optical rotatory power of proteins in different solvents put urea in a special category. Dill & Alsberg [1925] found that gliadin had a higher rotation in 30% urea than in various dilutions of ethyl and propyl alcohols, and Pauli & Weiss [1931] found that proteins in solutions of sodium benzoate and salicylate, which like urea are denaturing agents, had the same rotation as in water, whereas in urea solutions they had a higher rotation.

2. *The size and shape of proteins in urea solution*

Solution in urea seems to affect the osmotic pressure of individual proteins differently. Burk & Greenberg [1930] found that in urea solution horse haemoglobin had half its normal molecular weight, as measured by osmotic pressure, and edestin one-quarter. Burk [1937, 1] found similar changes in osmotic pressure with amandin and excelsin, and [1940] *Limulus* haemocyanin. Wu & Yang [1932] confirmed the fall in the molecular weights of ox and horse haemoglobins but found the values for sheep and dog haemoglobins to remain unchanged after treatment with urea, although Drabkin [1939] has shown that this treatment does denature dog haemoglobin. Hand [1935] claimed that the effects of hydration had been overlooked in these measurements on haemoglobins, but his criticism met with little support [cf. Steinhardt, 1938]. Similar changes have been recorded with the muscle proteins [Weber, 1933; Weber & Stover, 1933]; in 45% urea myosin had an apparent molecular weight of 10^5 instead of about 10^6 and myogen one of 34,000 instead of 81,000. On the other hand, serum albumin, investigated in some detail by Burk [1932] and Pauli [1934], behaved differently. Burk discussed the corrections that must be applied because of departures from the ideal solution law and concluded that serum albumin had the same molecular weight in urea solution after denaturation as it had in water or in 75% glycerol. Burk [1937, 2; 1938] also showed that serum globulin and gliadin were denatured by urea without changing molecular weights. Huang & Wu [1930] denatured egg albumin in different ways and found differences between the osmotic pressures in urea solution, but Burk & Greenberg [1930] and Burk [1937, 1] found the same osmotic pressure for egg albumin whether it was dissolved in water or in 40% urea. The fact that Williams & Watson [1937] found a sedimentation constant for egg albumin in 50% urea corresponding to a molecular weight of 21,000 instead of the normal value of 36,000 at first sight contradicts Burk's results. However, all these results assume, that the particles of both native and denatured protein are spherical, and Bull [1940] has pointed out that the increase in viscosity of egg albumin on denaturation is evidence that the particles are no longer spherical. This conclusion is compatible with the observation of Lee & Wu [1932] that the area per molecule of films of native and urea-denatured egg albumin, making the usual assumptions about molecular weight and density, are 8400 and 10,400 sq. Å., but this increase in area was not confirmed by Bull [1938].

Too few observations have been made on the viscosities of native and urea-denatured proteins to permit any valid generalizations, but there appears to be an interesting difference between initially anisodimensional and viscous proteins and the more mobile proteins [cf. Hand, 1935]. Liu [1933], Neurath & Saum [1939] and Bull [1940] found a rise in the relative viscosity of the globular egg albumin on denaturation, whereas Frampton [1939] and Edsall & Mehl [1940] found that denaturation increased the mobility of the rod-shaped proteins

tobacco mosaic virus and myosin. Many workers have noticed the increased fluidity given by urea to glutinous products, and this property was used by Dold [1924] for liquefying sputum as a preliminary to examination for tubercle bacteria.

3. *Evidence on the nature and reversibility of the changes wrought by urea*

It is clear that the action of urea on a protein is complex and that the strength of the urea, the time of action and the temperature may determine which of the possible actions occurs. The milder changes, such as swelling, increase in solubility and permeability, and perhaps the attachment of urea to parts of the protein, are generally reversible [Heim, 1937]. Carpenter & Lovelace [1938], from a study of the rotatory dispersion, found with gelatin that the effects even of concentrated urea were completely reversible, but this is exceptional and with most proteins intense treatments cause a change that may properly be regarded as denaturation [cf. Mirsky & Pauling, 1936]. This change is again complex and proceeds through a succession of stages according to the severity of the treatment with urea. For example, Beck & Schormüller [1937] in studying the solution of horse meat in urea found that heating solutions of the already denatured protein had a further effect in preventing its precipitation on dialysis. Diebold and Juhling [1938] obtained similar results with fibrinogen, and Steinhardt [1938] found that only 30% of a sample of horse haemoglobin precipitated on dialysis after exposure to 4 *M* urea whereas all precipitated after exposure to 7.46 *M*. Edsall & Mehl [1940], extending the observation of von Murralt & Edsall [1930] on the loss of anisotropy of flow of myosin in strong urea solutions, commented on the fact that this loss occurred when the treatment was insufficient to reduce the viscosity to its minimum value.

Hopkins [1930] first showed that proteins varied in their resistance to denaturation by urea. The denatured protein is usually recognized by diluting or dialysing solutions until the urea is too dilute to keep it in solution. Steinhardt [1938] and Burk [1937, 2] have stressed the uncertainty of this criterion, partly because of the solubility of denatured protein in the presence of native protein and partly because of the readiness with which the first stages of denaturation may be reversed by dialysis. Steinhardt also presented evidence that denaturation of some proteins did not occur in the urea, even though dissociation had taken place, but that it was a result of subsequent treatments such as dilution or dialysis. On the other hand, Burk [1932] found that the heat coagulation of serum albumin could be partly reversed by dissolving it in urea and dialysing at a low temperature; Laporta [1932] made similar claims for other proteins.

For obvious reasons experiments on proteins with measurable specific activities would give the most satisfactory evidence that urea has had an effect that is irreversible by dilution or dialysis. As yet few of these have been made. The concentrations of urea used in experiments with pepsin and trypsin have not affected them irreversibly. The behaviour of fibrinogen is interesting, for Wöhlisch & Kiesgen [1936] found that in 30% urea it could no longer be coagulated by heat, alcohol or thrombin, whereas Meissner & Wöhlisch [1937] recovered from the solution fibrinogen that could be clotted. Diebold & Jühling [1938] examined the system in greater detail, finding that thrombin could act in the presence of 15% urea but that the precipitation of fibrin was prevented. A water-soluble protein that could no longer be clotted with thrombin could be made by more vigorous treatment with urea. The effects of urea on the activities of viruses and bacteria are described later in this paper.

Steinhardt's [1938] hypothesis that urea and other amides act by competing with peptide bonds in one part of a protein for association with neighbouring bonds in the protein grid is compatible with the evidence so far adduced. On this hypothesis the protein is held together by these associations and the probability that a protein will return to its original configuration on the removal of urea will depend on the number of these associations that have been broken and have to reform. With conjugated proteins, urea may split off the prosthetic group. Experience shows that this usually leads to greater instability of the protein moiety, so that denaturation of that might also be expected. Some agents, e.g. sodium dodecyl sulphate [Sreenivasaya & Pirie, 1938] may dissociate a prosthetic group without denaturing the protein, but no example is known of urea acting in this way, although it is presumably possible. On the other hand, examples of denaturation without the liberation of the prosthetic group are known.

The most characteristic chemical difference between native and denatured proteins is the presence in the latter of free $-SH$ groups, or of groups that can readily be turned into $-SH$ groups by reducing agents. This effect was first demonstrated by Hopkins [1930] with egg albumin and horse and sheep serum proteins after denaturation by urea. Burk [1937, 1] confirmed these results and showed that amandin, edestin, excelsin, sheep haemoglobin and myogen also gave $-SH$ groups, but not gliadin, zein or pepsin. Quantitative measurements of the $-SH$ groups formed by action of urea and related substances have been made by Greenstein [1938; 1939] on egg albumin and by Greenstein & Edsall [1940] on myosin. Not all the available $-SH$ was liberated from myosin by prolonged treatment with urea, for on denaturation with guanidine twice the apparent cysteine content was found. Tobacco mosaic virus also gives an $-SH$ reaction after denaturation with urea. Little other chemical work has been done on differences between native and urea-denatured proteins. Chou & Wu [1936] found that it had no effect on the formaldehyde titrations of five proteins, suggesting that the $-NH_2$ groups were not concerned in the changes, although Hopkins [1930] and Wu *et al.* [1931] noticed a change in pH on denaturation.

4. *The effects of urea on bacteria and tissues*

We have already referred to the first experiments on tissues in which urea was used as a solvent, and we conclude this survey by describing some systems in which it is probable, though not certain, that the changes accompanying exposure to urea result from its action on protein. Mustard and cress seeds exposed to 5% urea did not germinate [Ramsden, 1902]; *Esch. coli* barely grew in media containing 8% urea [Wilson, 1906] and was more easily lysed by its bacteriophage in the presence of 5% urea [Bronfenbrenner & Hetler, 1933]. Changes to filamentous forms were described by Wilson [1906] and by Péju & Rajat [1906] when some bacteria were grown on media containing 2–5% urea, but many bacteria, e.g. staphylococci, streptococci and sarcinae, were apparently unaffected. Symmers & Kirk [1915] measured the rate at which *Ps. pyocyanea* was killed by the presence of urea when suspended in blood, and found 25 g. per 100 ml. to be the lowest effective concentration. They recommend the liberal use of urea in the treatment of wounds and mention the use of solutions as mouth washes for diphtheria carriers and for sterilizing tuberculous sputum. Individual bacteria differ widely in their resistance to concentrated urea solutions [Dold, 1924; Foulger & Foshay, 1935; Finger, 1937]; *Myc. tuberculosis* was the most resistant tested and was killed by 3 hr. exposure to saturated urea at 37°.

Bacterial spores were much more resistant than bacteria. Rooschütz [1935] noted their presence in most commercial samples of urea, and Dold & Weyrauch [1924], having found spores to be viable after a month's exposure to saturated urea at room temperature, recommended the use of urea solutions in their isolation.

In popular medicine the use of urine and of plant extracts containing related substances such as allantoin, e.g. comfrey [Macalister, 1912], for promoting wound healing is some thousands of years old. Solid urea was used to prevent the suppuration of wounds by Symmers & Kirk [1915], but no further use of the treatment seems to have been made until Millar [1933] described its beneficial effects on sloughing cancers. Since then the successful use of solid urea on a large number of wound cases has been described by Holder & MacKay [1937; 1939], by Muldavin & Holtzmann [1938] and others. Strong solutions seem to be equally effective against chronic infections of the ear, nose and throat [Foulger & Foshay, 1935; Mertins, 1937]. Treatment with solid urea resembles in some ways the well-known treatment of wounds with fly maggots. Presumably part of the benefit in both treatments comes from the removal of damaged tissue, by solution with urea and by selective feeding with the maggots. It is unknown why urea does not dissolve healthy tissues, but this relatively specific action has been noted by Stoeltzner [1925] and Stoye [1925], who claimed that the injection of concentrated urea solutions under non-adherent scar tissue led to its ready removal and to the smooth healing of the wound. They found, however, that adhering scar tissue was apt to become necrotic with this treatment. Tissues are readily permeable to urea, and it may be that where there is an undamaged blood supply, a sufficiently high concentration of urea to dissolve tissue proteins cannot be maintained. In wound healing, bacteriostatic action is doubtless of great importance, but it has also been suggested, notably by Robinson [1938], that urea has a directly stimulating action on cell proliferation. Mond & Hoffman [1928] found that urea as dilute as 0.3*M* damaged the surface of red blood corpuscles and tended to make lysis easy. Smadel *et al.* [1938] have also made similar observations, but this property does not seem to have caused any difficulties in the clinical use of urea.

5. *The effect of urea on viruses*

Burnet [1933] tested the resistance of 24 different dysentery coli bacteriophages to urea and divided them into three groups according to whether they were rapidly inactivated, slowly inactivated or unaffected by 27.7% urea; he suggested that the smaller phages were the more resistant. McKay & Schroeder [1936] found that rabies and anterior poliomyelitis viruses lost both their ability to infect and to immunize rabbits after exposure to 40% urea. Hoyt & Warner [1940] confirmed the inactivation of rabies virus, but found that after less severe treatment, so that it was not completely inactivated, it retained its immunizing power. Smadel *et al.* [1938] found that in 22% urea there was an initial rise in the sedimentation constant of vaccine virus followed by a fall, whereas in 40% the fall was continuous. Inactivation was complete in 3 days in 22% urea, whereas a 10–15% solution was without any apparent effect. These changes presumably correspond to the swelling that has been observed with some other proteins in dilute urea and with the denaturation that occurs in concentrated solutions. Höring [1939] showed that concentrated urea quickly inactivated yellow fever virus, whereas in dilute urea the virus was about as stable as it was in water.

Bawden & Pirie [1937] found that three strains of tobacco mosaic virus were more resistant to urea than were the proteins used by Hopkins [1930] or than some of the phages used by Burnet [1933]. No details were given, though the denaturation of the viruses and the loss of liquid crystallinity after several hours' exposure to saturated urea were mentioned. Mehl [1938] also mentioned the loss of anisotropy of flow of tobacco mosaic virus preparations after treatment with urea. Frampton & Saum [1939] and Frampton [1939] reported that solution of tobacco mosaic virus in 6 *M* urea and 0.1 *M* phosphate buffer caused a hundredfold increase in the diffusion constant with no change in infectivity. They interpreted this result as indicating that the urea caused the virus particles to disaggregate into single molecules of molecular weight around 100,000. They suggested that these were the true virus molecules and that the large particles with weights equivalent to molecular weights of many millions were merely aggregates. However, no increase in infectivity accompanied the urea treatment such as would be expected from such a disaggregation into small infective units, and later work has not confirmed this view. Stanley & Lauffer [1939] were unable to obtain any evidence that the low molecular weight protein produced by the action of urea was infective. They found that the disintegration depended on the salt content, *pH* and temperature, and any residual activity in treated virus preparations was always found to be associated with remaining protein of high molecular weight. Measurements in the ultracentrifuge [Martin, 1939] indicated that the inactivated virus had a molecular weight of 400,000, but osmotic pressure measurements [Stanley & Lauffer, 1939] showed that on continued action of urea it fell to 40,000.

EXPERIMENTAL

1. *Materials and methods*

In this paper experiments are described with the four viruses, tobacco mosaic, tomato bushy stunt, potato "X" and tobacco necrosis. The virus preparations used were made by the methods described previously [Bawden & Pirie, 1937; 1938, 1; 1938, 2; Pirie *et al.* 1938]. The tobacco necrosis virus used probably differs from that used earlier, for although the preparations used were made by the methods described then we have been unable to get any crystalline material. Instead, the whole preparations have consisted of amorphous material closely resembling the amorphous two-thirds of the previous preparations. The serological reactions of our preparations also differ from those of the earlier preparations. It seems probable that the virus culture used in 1938 was a mixture of strains, of which only one was crystallizable, and that in repeated transfers during the last two years this strain has been lost.

Because of shortage of material only a few tests were made with potato virus "X" and tobacco necrosis virus. Most were made with tobacco mosaic virus, because it is both readily obtained in large quantities and shows the convenient property of anisotropy of flow. Qualitative observations on the loss or diminution of this property, made by shaking solutions in test tubes, 0.5–1 cm. in diameter, between crossed polarizers, give valuable preliminary indications of the severity of treatment necessary to disintegrate the virus. This provides quite a sensitive test, for the disintegration products not only themselves fail to show anisotropy of flow but also impede the orientation of the still undamaged virus so that it will no longer form a liquid crystalline layer and so that a greater rate of shear is necessary to get maximum orientation. Some treatments destroy the infectivity of tobacco mosaic virus without disintegrating

it. These treatments also leave the anisotropy of flow and serological reactions unimpaired, but urea has not been found to do this.

For simplicity the purified materials used in our tests will be called viruses, although their exact relationships with the viruses as they occur in infective saps is still uncertain. With tobacco mosaic virus and potato virus "X" it is known that purification by the methods used leads to a reduction in infectivity and to some other changes, which have been attributed to the linear aggregation of virus particles [Bawden & Pirie, 1937; 1938, 1, 2, 3]. Except for tobacco necrosis virus, which was tested only for changes in infectivity, the treated virus preparations were tested both for their infectivity and serological activity. In the infectivity tests dilutions were made in 0.1 *M* phosphate buffer at pH 7, and local lesion counts were made at two dilutions of the inocula, usually at 10^{-4} and 10^{-5} g. protein per ml. The host used for lesion counts for tobacco mosaic and tomato bushy stunt viruses was *Nicotiana glutinosa*, for potato virus "X" *N. tabacum*, var. White Burley, and for tobacco necrosis virus *Phaseolus vulgaris*, var. Canadian Wonder. To reduce to a minimum the errors arising from differences in the susceptibility of individual plants, infectivity tests were all arranged in the form of Latin squares or incomplete blocks [Youden, 1937]. In each test at least six leaves were inoculated with each dilution of the preparation tested. As solutions containing the same quantities of active virus can often give widely different numbers of local lesions if they contain different amounts of salts or other materials, the compositions of all test and control inocula were adjusted to be at the same pH, and to contain the same amounts of urea, salts and protein before inoculation.

The dilutions in the serological tests were made in 0.85% NaCl solution. 1 ml. of antiserum at a dilution of 1/50 was added to a series of tubes each containing 1 ml. of virus solution at different concentrations, and the smallest amount of virus in g. to give a precipitate visible to the eye in 2 ml. of such mixtures is recorded as the serological titre. The serological titres of different preparations of tomato bushy stunt virus are remarkably constant, but different preparations of tobacco mosaic virus and of potato virus "X" give widely different titres. The titre of any one preparation remains constant and reproducible, changes in it accurately reflecting changes in the virus. Therefore, as the tests described were carried out over a long period and with different virus preparations, although the serological titres given in any one table are strictly comparable, they cannot necessarily be compared with those in the other tables or with those previously published.

2. Effect of different concentrations of urea

It is well known that the rate at which a disinfectant works is a non-linear function of its concentration [Smith, 1921], and that below a critical concentration it has no appreciable effect. This is also true for the killing of bacteria by urea, for which Wilson [1906] and Symmers & Kirk [1915] have found the critical range of concentration to be from 8 to 25%, i.e. from 1.33 to 4.17 *M*. Tables 1-4 show the effects of variations in the urea concentration on the rate of inactivation of the four viruses. They show clearly that concentrated urea has had an effect on the specific activities of all the viruses that is not reversed by dilution. They also show that the individual viruses differ widely in their resistance to the inactivating action of urea, and that, as with bacteria, there is for each one a critical concentration below which there is little or no inactivation. With all the viruses, the reduction in infectivity is approximately proportional to the reduction in serological activity, and with none is there any indication

that the proteins with low molecular weights produced by the action of urea on the large virus particles have any specific virus activities as suggested by Frampton & Saum [1939] for tobacco mosaic virus. In establishing the lack of action, or more probably the reversibility of the action, of dilute urea on the viruses the results also show that it is legitimate to study the kinetics of the action by diluting the mixtures largely when the action is to be stopped.

Table 1. *Effect of concentration of urea on inactivation of tobacco mosaic virus*

Urea <i>M</i>	ml. of phosphate and urea mixed to get molarity		ml. of phosphate and urea added in diluting		Serological titre	Average no. of lesions at	
	Phosphate	Urea	Phosphate	Urea		10 ⁻⁴	10 ⁻⁵
8	0.0	0.8	2.0	0.0	1/5000	1	0
7	0.1	0.7	1.9	0.1	1/10,000	2	0.5
6	0.2	0.6	1.8	0.2	1/40,000	15	3
5	0.3	0.5	1.7	0.3	1/80,000	24	5
4	0.4	0.4	1.6	0.4	1/320,000	65	28
3	0.5	0.3	1.5	0.5	1/640,000	116	44
Control; urea diluted before adding virus					1/640,000	117	54

To 0.2 ml. of 1.64% tobacco mosaic virus was added sufficient *M*/10 phosphate at pH 7.1 and 10 *M* urea solution to give 1 ml. of solution at the required molarity. After 20 hr. at 15° each was diluted with phosphate and urea to a final volume of 3 ml., i.e. to 2.67 *M* urea, and tests were made 36 hr. later.

Table 2. *Effect of concentration of urea on inactivation of tomato bushy stunt virus*

Urea <i>M</i>	Serological titre	Average no. of lesions per leaf at	
		10 ⁻⁴	10 ⁻⁵
8	No ppt. at 1/5000	0	0
7	1/10,000	12	6
6	1/160,000	108	47
5	1/640,000	137	82
Control	1/640,000	157	79

The samples were prepared as in Table 1 and were exposed for 20 hr. at pH 8 and 15°. They were then diluted till the urea was 1.6 *M* and tested 36 hr. later.

Table 3. *Effect of concentration of urea on inactivation of potato virus "X"*

Urea <i>M</i>	Time	Serological titre	Average no. of lesions per leaf at	
			10 ⁻⁴	10 ⁻⁵
7	10 min.*	No ppt. at 1/10,000	0	0
6	1 hr.*	No ppt. at 1/10,000	1	0
4.5	1 hr.*	No ppt. at 1/10,000	6	1
3	1 hr.*	1/40,000	63	15
1.2	36 hr.	1/320,000	133	110
Control		1/640,000	220	190

* These preparations spent an additional 36 hr. exposed to 1.2 *M* urea.

In all tests 0.2 ml. lots of 0.48% salt-free potato virus "X" were diluted with the volumes of water and 10 *M* urea in 0.1 *M* phosphate buffer at pH 7.1 required to give 0.5 ml. at the required molarity of urea. After the stated time at 17° they were diluted to 1.2 *M* urea and tested 36 hr. later.

ACTION OF UREA ON VIRUSES

Table 4. *Effect of concentration of urea on the inactivation of tobacco necrosis virus*

Urea <i>M</i>	Average no. of lesions per leaf at	
	10 ⁻⁴	10 ⁻⁵
7.5	0	0
5.5	10	2
3.5	82	20
Control	240	130

The samples were exposed for 20 hr. at pH 7 and 16°.

3. *Effect of temperature on the rate of inactivation*

The fact that proteins have a large temperature coefficient of denaturation makes Hopkins's [1930] discovery that the denaturation of egg albumin in the presence of urea proceeds faster at 0° than at 37° exceptionally interesting. Stanley & Lauffer [1939] found that the disintegration of tobacco mosaic virus in urea proceeded more rapidly at 0° than at 25°, but that it proceeded more rapidly at 40° than at 25°. We have confirmed this result with tobacco mosaic virus, and have found that potato virus "X" and tomato bushy stunt and tobacco necrosis viruses also have temperature ranges in which cooling leads to an increase in the rate of denaturation by urea. Table 5 shows the results of an

Table 5. *Effect of temperature on the denaturation of tobacco mosaic virus by urea*

Temperature	Serological titre	Average no. of lesions per leaf at	
		10 ⁻⁴	10 ⁻⁵
0°	1/100,000	13	4
9.5	1/400,000	20	6
20	1/800,000	31	7
37	1/200,000	6	2
55	No ppt. at 1/50,000	0	0
Control	1/1,600,000	40	12

Each sample, except the control, was exposed to 6*M* urea for 4 hr. in the presence of *M*/30 phosphate buffer at pH 7.0 and the temperature stated, they were then diluted for testing. The control was diluted immediately after mixing at 15°.

experiment on the denaturation of tobacco mosaic virus by urea between 0° and 55°, and Table 6 those for a more detailed experiment between -10° and +11°. Results of experiments at different temperatures with potato virus "X" and tomato bushy stunt virus are given in Tables 7 and 8.

The viruses used in these temperature tests vary widely in their reactions to freezing and drying, presumably because they hold water in different ways. Bushy stunt virus in salt-free solution is denatured and inactivated by freezing and thawing, whereas tobacco mosaic virus and potato virus "X" are not, and bushy stunt virus and potato virus "X" are completely inactivated when neutral solutions are dried, whereas tobacco mosaic virus is only partially inactivated. In spite of these differences in the properties of the individual viruses, the denaturation of all of them by the urea shows a similar increase in rate at low temperatures. The remote possibility exists that exposure of virus solutions to low temperatures alone might have some effect; for example, exposure to -10° might inactivate tobacco mosaic virus even though freezing at

Table 6. *Denaturation of tobacco mosaic virus by urea between -10° and 11°*

Urea <i>M</i>	Time of exposure hr.	Temperature	Serological titre	Average no. of lesions per leaf at	
				10 ⁻⁴	10 ⁻⁵
6	24	11°	1/50,000	3.1	0.8
		0	1/20,000	2	0.2
		- 5	1/10,000	0.5	0.1
		-10	No ppt. at 1/5000	0	0
	48	11	1/40,000	5.2	0.6
		0	1/10,000	0.7	0
		- 5	1/5000	0.1	0
		-10	No ppt. at 1/5000	0	0
5	24	11	1/200,000	30.7	6.6
		0	1/100,000	18.4	2.5
		- 5	1/20,000	3.6	0.4
		-10	1/8000	0.8	0.2
	48	11	1/100,000	14.1	0.5
		0	1/80,000	8.5	1.4
		- 5	1/20,000	6.0	0.5
		-10	1/5000	1.7	0.1
		Control; urea diluted before adding virus	1/800,000	67.1	32.3

0.2 ml. samples of a 1.64% solution of tobacco mosaic virus were added to 0.8 ml. of 7.5 or 6.25 *M* solutions of urea in *M*/30 phosphate buffer at pH 7 and already at the temperature stated. After the stated time at the given temperatures, those exposed to 6 *M* urea were diluted with 2 ml. of water and those exposed to 5 *M* with 1.9 ml. of water and 0.12 ml. of 10 *M* urea. After 36 hr. at room temperature they were diluted further for testing.

Table 7. *Effect of temperature on the inactivation of potato virus "X" by urea*

Urea <i>M</i>	Temperature	Serological titre	Average no. of lesions per leaf at 10 ⁻⁴
3	37°	1/40,000	15
	10	1/80,000	25
	-10	1/20,000	10
2	37	1/160,000	43
	10	1/320,000	101
	-10	1/30,000	12
Control; urea diluted before adding virus		1/640,000	176

Samples of 0.24% solution of potato virus "X" exposed in *M*/30 pH 7.0 phosphate buffer at the temperatures stated for 1 hr. to the stated concentration of urea. Samples were then diluted to 0.5 *M* urea and left for 36 hr. at room temperature before testing.

Table 8. *Effect of temperature on inactivation of tomato bushy stunt virus by urea*

Temperature	Serological titre	Average no. of lesions per leaf at	
		10 ⁻⁴	10 ⁻⁵
37°	1/160,000	71	30
20	1/320,000	135	58
0	1/80,000	35	11
-12	1/20,000	0	0
Control; urea diluted before adding virus	1/320,000	145	80

Samples were exposed for 7 hr. in 6 *M* urea at pH 7.9 at the stated temperatures. They were then diluted to 2 *M* urea and left for 36 hr. at room temperature before testing.

this temperature does not. To test this possibility eight samples of 0.47% tobacco mosaic virus solution in pH 6.9 phosphate:borate buffer mixture [Kolthoff, 1925] were mixed with 2 vol. of either 6M urea, 6M acetamide, 6M formamide or 6M alcohol. One of each pair of these mixtures was kept at -10° and the other at 16° . All were then diluted until the urea or other substance was 1.5M, and they were tested 36 hr. later. The serological titres of the two samples containing urea were 1/10,000 for that kept at -10° and 1/640,000 for that kept at 16° , whereas all the other samples gave a titre of 1/2,560,000. Similarly, the effect of freezing solutions of tomato bushy stunt virus is a direct effect of the freezing and not one of the low temperature, for when infected leaves, infective sap or solutions of purified virus containing salts, are cooled below 0° the virus is not inactivated. The increased rate of denaturation at low temperatures seems to be an effect specific to urea, for other denaturants have been tested over a range in temperature and none has been found that inactivates more rapidly at -10° than at higher temperatures.

4. *Effect of pH changes on the rate of inactivation*

Hopkins [1930] showed that egg albumin was denatured in the pH range 5-6.5, but he made no detailed tests on the rate of denaturation by urea at different hydrogen ion concentrations. Stanley & Lauffer [1939] found that there was little or no denaturation of tobacco mosaic virus by 6M urea at pH 5.5, that it was more rapid at pH 8.2 than at pH 7.4 and that it was much slower at pH 6.4 than at pH 7.4. We have confirmed the effect of alkali in increasing the rate of inactivation of tobacco mosaic virus by urea, and have found tomato bushy stunt virus to behave similarly.

The pH of a buffer solution in the presence of concentrated urea is a matter of some uncertainty. Burk & Greenberg [1930] found that in 6.66M urea the apparent *pK* of acetic acid shifted from 4.6 to 5.2 and that of phosphoric acid from 6.8 to 7.2. Hopkins [1930] noticed an increase of 0.8 to 1.0 units in the pH of protein solutions on the addition of urea. There are probably at least three causes of this effect: (1) the *pK* shift noticed by Burk & Greenberg, (2) the pH shift of protein solutions when denatured [Wu *et al.* 1931] and (3) the presence of ammonium salts in most samples of urea and the gradual production of more of these on standing, especially if the solution is heated [Lewis & Burrows, 1912; Ogiu & Pauli, 1932; Beck & Schormüller, 1937]. In our experiments two methods were adopted for measuring the pH. In the first, 10M urea in M/10 phosphate buffer was added in suitable quantities to the virus solutions and the pH recorded by the hydrogen electrode after the mixture was diluted 10-fold (the apparent pH before such dilution was about 0.2 units higher). In the second, a virus: buffer mixture of the required pH value was mixed with a neutral solution of urea.

Table 9 shows the results of one experiment on the effect of pH on the inactivation of tobacco mosaic virus by urea. The critical range of pH within which the rate of inactivation of tomato bushy stunt virus increases rapidly is even narrower than that with tobacco mosaic virus, and is shown in Table 10. In this table, and in Table 8, it appears at first sight that urea treatment is destroying infectivity without proportionally affecting the serological activity. However, we have already shown [Bawden & Pirie, 1938, 2] that this is one of the effects of treating bushy stunt virus with alkali alone, although then it occurs only at a higher pH. Thus it seems that in the presence of urea this type of inactivation can occur in less alkaline solutions, but the effect is partially masked by secondary reactions that destroy both infectivity and serological activity.

Table 9. *Effect of changes in pH on the inactivation of tobacco mosaic virus in the presence of urea*

Urea <i>M</i>	<i>pH</i>	Serological titre	Average no. of lesions per leaf at	
			10 ⁻⁴	10 ⁻⁵
6	5.16	1/2,560,000	220	98
6	6.07	1/1,280,000	160	48
6	6.76	1/160,000	50	8
6	7.87	No ppt. at 1/1000	0	0
6	9.16	No ppt. at 1/1000	0	0
4	9.16	1/4000	3	1
0	9.16	1/64,000	180	80
Control; urea diluted before mixing		1/2,560,000	260	140

The samples were exposed for 20 hr. at 15°. 0.1 ml. of a 3.3% solution of tobacco mosaic virus solution was mixed with 0.3 ml. of a mixture of *M*/10 phosphate and *M*/20 borate made up according to Kolthoff [1925] to the *pH* stated. 0.6 ml. of 10 *M* urea was added to the top 5 samples, 0.4 ml. plus 0.2 ml. of water to sample 6 and 0.6 ml. of water to the last sample. After 20 hr. the requisite amount of borate or phosphate was added to each sample to bring the *pH* to 6.76, and water and urea were added to the last 2 samples to bring the volume to 3.35 ml. and urea concentration to 1.79 *M*. Tests were made 36 hr. later.

Table 10. *Effect of pH on the inactivation of tomato bushy stunt virus in the presence of 7.7 M urea*

<i>pH</i>	Serological titre	Average no. of lesions per leaf at	
		10 ⁻⁴	10 ⁻⁵
7.0	1/320,000	71	16
8.1	1/40,000	0	0
9.16	No ppt. at 1/10,000	0	0
Control; urea diluted before adding virus	1/320,000	55	15

0.15 ml. lots of Kolthoff buffer at the *pH* stated and containing 1 mg. of bushy stunt virus were mixed with 0.5 ml. of neutral 10 *M* urea. After 80 min. at 18.5° 2 ml. of water and 0.15 ml. of *M* acetate buffer at *pH* 4 were added, and tests were made after 36 hr.

5. Comparison of purified virus with clarified infective sap

The experiments already described were made with purified viruses prepared by precipitation methods. It is known with potato virus "X" and tobacco mosaic virus that these methods cause a fall in infectivity and filterability, and we have suggested [Bawden & Pirie, 1937] that this arises from an aggregation of the virus into particles of a greater length/width ratio. The most plausible explanation for this is that the virus particles as they occur in the plants have materials at their ends which keep them from aggregating, and that during the purification processes these end materials are removed. In view of the known differences between tobacco mosaic virus in infective sap and in the purified state, Martin's [1939] statement that the former is more readily inactivated by urea is of some interest. Martin has published no details of his experiments and we have therefore been unable to duplicate them, but in the tests we have made comparing the behaviours of crude and purified preparations the increased stability of the latter is only slight.

Leaves from infected tobacco plants were frozen and minced, and the sap expressed through muslin and centrifuged. The *pH* of the supernatant fluid,

measured by the hydrogen electrode, was 5.9. A 4% solution of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ was made with this sap, which, after centrifuging off the precipitate formed, had pH 6.9. As infective sap usually contains 1–2 g. of virus per litre, a comparison solution was made containing 1.57 g. of purified virus per litre in *M*/10 phosphate buffer at pH 6.9. Both solutions were then made up to 6*M* urea by the addition of solid urea, and after the required times at 15° samples were taken and diluted to 2*M* urea for testing later. To obtain 100 ml. of 6*M* urea, 73.5 ml. of solution were added to 36 g. of urea, so that the final concentration of purified virus in the mixtures was 1.57×0.735 , or 1.15 g. per litre. To the control solutions only sufficient urea was added to give a concentration of 2*M*. Table 11 gives the

Table 11. *Comparison of the rates of inactivation of purified and crude tobacco mosaic virus preparations at pH 6.9 in 6*M* urea*

Time	Phosphate: infective sap mixture Serological titre	Lesions		0.115% solution of purified virus in phosphate buffer		
				Serological titre	Lesions	
		10 ⁻⁴	10 ⁻⁵		10 ⁻⁴	10 ⁻⁵
30 min.	1/81	43	14	1/243	144	64
2.5 hr.	1/27	6	1.5	1/27	36	14
20 hr.	0	0	0	1/3	3	0
Control	1/729	215	158	1/2187	204	132

Description in text.

results of this comparison. It again illustrates the greater infectivity for a given serological titre that is characteristic of unpurified virus. It also shows that the inactivation of the virus in infective sap proceeds somewhat faster and farther than that of the purified virus, but the difference is slight and may well be caused by differences in the salt contents of the two preparations and by the action of the urea on the salts. Alternatively, it is possible that at the same time as the urea is denaturing the purified virus it also dissociates the aggregates formed during the purification processes, and that this slight increase in the number of infective units partly masks the inactivating effect and accounts for the slower inactivation of the purified virus.

6. *Chemical and physical changes accompanying inactivation by urea*

No detailed study has been made of the changes taking place during the inactivation of the viruses by urea, but the two anisotropic viruses with low nucleic acid content behave differently from the two isotropic viruses with higher nucleic acid contents. Stanley & Lauffer [1939] found that the inactivation of tobacco mosaic virus by urea led to a separation of the nucleic acid from the protein moiety. We have confirmed this, and find that nucleic acid is also liberated from potato virus "X" during the inactivation. On the other hand, the precipitates that separate from preparations of tomato bushy stunt and tobacco necrosis viruses denatured by urea still contain nucleic acid.

The solvent action of urea on native and denatured proteins was described in the introduction. The interrelation of these two actions with fibrinogen was studied by Djebold & Jühling [1938], who found that after a few hours at 37° solutions in 0.9% NaCl had a minimum opacity when they contained 12% of urea and a maximum when they contained 20%. Below 12% the lyotropic effect of urea on native fibrinogen was being measured and above 20% its solvent action on denatured fibrinogen, while between these two concentrations

denaturation and partial precipitation were proceeding. In their behaviour towards urea bushy stunt and tobacco necrosis viruses to some extent resemble fibrinogen. Tomato bushy stunt virus is almost completely inactivated by exposure to 7*M* urea for 20 hr. at pH 8 and room temperature, but at this urea concentration, in the presence of a trace of salt, the denatured protein is insoluble, and it dissolves only slowly in 8–9*M* urea. On the addition of a trace of salt to solutions exposed for 10–20 hr. to 6–8*M* urea there is an immediate, apparently irreversible precipitation. These precipitates, like the original virus, contain 5% of carbohydrate as estimated by heating with orcin and sulphuric acid. The carbohydrate can be removed from the denatured protein by extraction with boiling dilute ammonium hydroxide, as it can from virus denatured by heating or drying [Bawden & Pirie, 1938, 2]. Tobacco necrosis virus behaves similarly but the effect is more easily demonstrated as inactivation occurs with more dilute urea. The precipitate of denatured virus is also more readily soluble in urea, so that there is an immediate disappearance of the faint opalescence characteristic of the virus preparation when enough urea is added to a 0.12% virus solution to make it 8*M*. In more dilute urea solutions, from 4 to 6.5*M*, there is rapid precipitation, but after some hours the precipitated material dissolves. On diluting any of these solutions of inactivated tobacco necrosis virus with 0.9% NaCl solution a precipitate separates that still contains nucleic acid.

The absorption spectra of the more highly purified virus preparations have all shown a characteristic maximum at about 260 $m\mu$, i.e. in the region associated with absorption by nucleic acid. As part of a general examination of the manner of linkage of the nucleic acid to the protein moiety we have compared the absorption spectra of the separated protein and nucleic acid with that of the intact virus. These experiments have shown that processes leading to separation of the nucleic acid lead to diminution of the absorption in the ultra-violet. This result is not unexpected, for it is well known that the intensities, and even the position of, the absorption maxima of purines and pyrimidines are affected by substitution or by changes in ionization [cf. Ellinger, 1938]. Furthermore, the treatments effecting the separation increase the clarity of the fluids, presumably because the average molecular weights of the dissolved materials are less, and this reduces the amount of light scattered, especially in the ultra-violet range. It is reasonable to suppose that much of the apparent absorption by plant virus preparations in the ultra-violet is caused by scattering. Lavin *et al.* [1939] found that tobacco mosaic virus preparations made by precipitation with ammonium sulphate absorbed more strongly than those made by ultra-centrifuging, and, as the former are the more highly aggregated, part at least of this effect may be caused by the difference in scattering.

Urea dissociation of potato virus "X" illustrates the effect on the absorption spectrum of the separation of the nucleic acid from the protein and the breakdown of the protein into smaller particles. When we described the purification of virus "X" [Bawden & Pirie, 1938, 1] we did not publish a spectrum, but in a later paper [Bawden & Pirie, 1939] preparations were described that had no absorption minimum at 245 $m\mu$. By repeated differential high speed centrifuging of chemically purified products we now get preparations with a minimum at 245 $m\mu$, although it is not so pronounced as those found with tobacco mosaic, tomato bushy stunt or tobacco necrosis viruses. This agrees with the figures published by Lavin *et al.* [1939]. The curves in Fig. 1 give the density of a 2 cm. layer of a 0.029% solution of potato virus "X" in the presence of 0.77*M* urea and 0.08*M* phosphate buffer at pH 7. For the upper curve, A, the virus was added to urea at 0.77*M* and the spectrum taken immediately. For the lower

curve, *B*, the solution was made by adding the virus to a concentrated urea : phosphate mixture so that it was exposed to 7.1 *M* urea. After 40 min., sufficient time for complete inactivation, the mixture was diluted to 0.77 *M*. Short exposure to 0.77 *M* urea apparently had no effect on the virus, for the curve *A* was identical with that given by the virus dissolved in water.

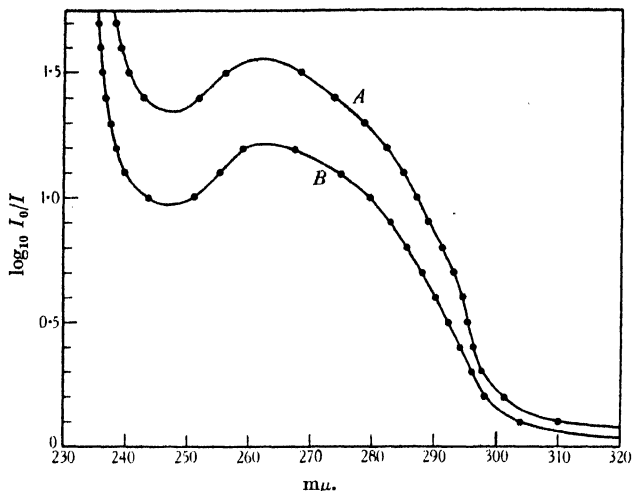


Fig. 1.

Changes similar to those with potato virus "X" occur when tobacco mosaic virus is inactivated with urea. On the other hand, absorption in the ultra-violet by tomato bushy stunt preparations is increased by denaturation with urea. The protein and nucleic acid are not separated when bushy stunt virus is treated with urea and the inactivated virus is insoluble in urea. Either of these differences between bushy stunt and the other two viruses may explain the difference in the behaviour of its absorption spectrum, for although in the absence of salts there is no actual precipitation of denatured protein, the fluid becomes more opalescent. When bushy stunt virus is inactivated by sodium dodecyl sulphate the protein and nucleic acid are separated and remain water-soluble, and there is an increase in the clarity of the solution. After this treatment the intensity of absorption of ultra-violet is increased, so that from this point of view the inactivation of bushy stunt by sodium dodecyl sulphate resembles the inactivation of tobacco mosaic virus and of potato virus "X" by urea.

DISCUSSION

In the experiments described in this paper urea has been regarded as the chief agent in causing denaturation. However, as denaturation requires the simultaneous control of several variables, this distinction is arbitrary. For example, as the rate of denaturation closely depends on the *pH*, it is as logical to regard urea as increasing the rate of alkaline inactivation as it is to regard alkali as increasing the rate of urea denaturation. Actually there is probably considerable interaction between the various factors. For example, our results show that at some *pH* values in the alkaline range, which do not themselves inactivate tobacco mosaic virus, concentrations of urea, which in neutral

solution do not inactivate, cause rapid inactivation. Also, one of the features of alkaline inactivation of tomato bushy stunt virus, loss of infectivity without loss of serological activity, occurs in less alkaline solutions in the presence of urea. Similarly, Drabkin [1939] compared the rates of denaturation of haemoglobin in dilute alkali, in 6*M* urea, and in a mixture of the two, and found that the mixture acted 60 times as rapidly as either component alone.

The restriction of the work reported to inactivation in the presence of urea is also largely arbitrary, for it is reasonable to assume that urethane, guanidine and related substances act in a similar manner and that there is a smooth transition to the somewhat remote denaturing agents such as pyridine, benzoates, salicylates, phenol and soaps. The effects of these substances on the plant viruses will be described in a later paper, but as little or nothing is known of the mechanism of their action it would be premature to attempt to classify them. There is one feature of inactivation in the presence of urea, however, that might justify the separation of urea from other denaturing agents and justify the view that urea is the chief agent in causing the denaturation rather than the other factors such as pH. This is the large increase in the rate of denaturation produced by cooling at temperatures below about 20°. This was found to be true for all the viruses we have tested, as well as for other proteins, but no such effect has been found for inactivation by alkali or other denaturing agents.

In our experiments only changes irreversible on dilution or dialysis and resulting in loss of specific virus activities have been investigated. The results show that for each virus there is a critical concentration of urea below which irreversible changes do not occur. This concentration varies with the different viruses, which, although they are all nucleoproteins, also differ in the manner in which they break down on denaturation. For example, the inactivation of potato virus "X" and tobacco mosaic virus is accompanied by the separation of the nucleic acid from the protein and the products are soluble in urea solution, whereas the inactivated bushy stunt and tobacco necrosis viruses are insoluble in urea and the precipitates still contain nucleic acid. In addition to these irreversible changes it is probable that urea can cause changes that are readily reversed and have no effect on virus activity. Frampton [1939] found that the addition of as little as 1*M* urea to solutions of tobacco mosaic virus greatly reduced the viscosity. As inactivation and disruption of the virus particles occur only slowly in much more concentrated urea solutions, it is probable that the immediate effect on viscosity is caused by the hydration of the particles and not by denaturation. We have found no evidence that the small particles produced by the disruption of the viruses possess virus activity, although the fact that purified virus is inactivated rather more slowly than impure virus may be evidence that the urea can dissociate the aggregates formed during purification as well as disrupt the actual virus particles.

SUMMARY

The literature on the effects of urea on proteins, tissues, bacteria and viruses is reviewed. The four viruses, tobacco mosaic, potato "X", tomato bushy stunt and tobacco necrosis, are irreversibly denatured by urea. The denaturation is accompanied by loss of infectivity and serological activity. For each virus there is a critical concentration of urea below which there is no irreversible effect on infectivity. This concentration is smallest for potato virus "X" and greatest for tomato bushy stunt virus. The rate of inactivation is greatly increased by the presence of alkali. The rate of inactivation is minimum at about 20° and is much increased by cooling to -10°. The inactivation of purified tobacco mosaic

virus by urea proceeds only slightly more slowly than that of virus in crude infective sap. The inactivation of tobacco mosaic virus and potato virus "X" is accompanied by separation of the nucleic acid and protein, but the inactivation of bushy stunt and tobacco necrosis viruses is not. Changes in the absorption spectra that accompany inactivation are described.

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151. THE EFFECTS OF ALKALI AND SOME SIMPLE ORGANIC SUBSTANCES ON THREE PLANT VIRUSES

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INTRODUCTION

At the end of last century it was widely recognized that many substances, notably choline [Mauthner, 1874], sodium myronate and the mustard oils, could dissolve otherwise insoluble proteins, and this property was applied clinically by some workers. Hebra [1892] and Mendel [1905] recommended thiosinamine (sold dissolved in sodium salicylate under the name "Fibrolysin") for the softening and ultimate removal of scar- and fibrotic-tissue, and Fraenkel [1915] also recommended choline for this purpose. The clinical use of urea, to which we have previously referred [Bawden & Pirie, 1940], seems to have been developed independently of these observations. Spiro [1900] quoted many of the scattered observations of the early workers and described the solvent action, demonstrated either by dissolving a coagulum or by raising the temperature of heat coagulation, of urea, choline, piperidine, pyridine, urethane, formamide and mustard oils. He concluded that some of these acted simply as bases but that others formed compounds with the proteins. He [1900; 1904] also observed that some of these agents were protein precipitants when dilute although they were solvents when concentrated. A similar effect was noticed by von Furth [1896] during a study of the coagulation of muscle and serum proteins by salicylate and several alkaloids and it has been observed and commented on by many workers since. Neuberg [1916] studied the effects of salts of 43 organic acids, mainly aromatic, on the solubilities of casein and several other substances normally difficult to dissolve, and he found that benzoates, hippurates and many substituted benzoates increased solubility greatly. Recent papers have tended to be less extensive, concentrating on the action of one substance or on a group of closely related ones. Stoeltzner [1925], Hopkins [1930], Pauli & Weiss [1931], and Edsall & Mehl [1940], however, have compared the actions of a wide range of substances on a small number of test objects.

Svedberg and his colleagues studied protein solutions containing protamines, arginine or lysine, in the ultracentrifuge, and found that the proteins were often dissociated into apparently homogeneous particles of smaller molecular weights. This was a valuable confirmation and extension of the osmotic pressure measurements made on proteins dissolved in urea and acetamide. Details of the experiments have not been published [cf. Svedberg & Pedersen, 1940], but from the published summaries [Svedberg, 1937; Lundgren, 1938; Pedersen, 1938] it is clear that there was considerable specificity of action. For example, in the

presence of ammonium chloride arginine dissociated serum albumin but not *Helix* haemocyanin, whereas lysine dissociated the haemocyanin but not the albumin, and neither acted without the ammonium chloride.

In the experiments described in this paper purified preparations of the three plant viruses, tobacco mosaic, tomato bushy stunt and potato "X", were used. The methods of preparation and of testing were the same as in the experiments with urea [Bawden & Pirie, 1940]. For a number of reasons the results obtained with these purified virus preparations may differ from those that might have been obtained with crude preparations. First, the viruses may have undergone physical or chemical changes during purification. Secondly, the large amounts of normal plant proteins and other materials in infective saps might remove the agents added and so protect the viruses from their effects. Thirdly, concentrations of the agents too dilute to affect the viruses directly may produce a coagulum of normal plant protein and still active virus may be adsorbed on to and removed by this.

EXPERIMENTAL

Sodium dodecyl sulphate and alkali

The hydrogen sulphate of dodecyl alcohol (S.D.S.) was used by Bawden & Pirie [1938, 1, 2] to inactivate potato virus "X" and tomato bushy stunt virus and it was found to separate the nucleic acid from the proteins. The work was extended to tobacco mosaic virus by Sreenivasaya & Pirie [1938], who made a more detailed study of the nucleic acid and the water-soluble, phosphorus-free protein produced by its action on this virus. Of the three viruses, potato virus "X" is the most susceptible to the action of S.D.S. and tomato bushy stunt virus the least. Even potato virus "X", however, is more resistant than other proteins that have been tested. S.D.S. has little effect on this virus at dilutions greater than 0.3% in neutral solution, although changes in haemoglobin [Anson, 1939], phytylchlorin [Smith, 1940] and cytochrome *c* [Keilin & Hartree, 1940] have been brought about rapidly at 10 times this dilution. Analogous changes are caused by some other surface active substances, e.g. soaps and bile salts, and these have been discussed in the papers quoted.

Sreenivasaya & Pirie [1938] found that tobacco mosaic virus was almost inactive after 24 hr. in 1% S.D.S. at 37° and pH 8. If either the temperature or the pH is raised the inactivation proceeds more rapidly and more dilute S.D.S. can be used, but the effect is complicated by the inactivation caused by the heating or alkali alone. At either room temperature or 37° the products of alkaline inactivation of tobacco mosaic virus remain soluble in the presence of small amounts of salts, although they are readily coagulated by strong salt solutions. At 55° or higher, however, the protein precipitates as it is liberated from combination with the nucleic acid unless the solution is more alkaline than pH 11. In tests in which tobacco mosaic virus at pH 10.14 was heated for varying periods at 55°, the falls in infectivity and serological activity were found to be proportional to the weight of protein precipitated, and this weight when plotted against the log of time gave a straight line. After 160 min. the preparation was completely inactivated, and 87% of the starting material was precipitated as a carbohydrate-free protein, a figure agreeing well with the expected content of protein free from nucleic acid. As our results on the increase in the rate of alkaline inactivation of tobacco mosaic virus by heat, which may equally well be regarded as a reduction in the thermal inactivation point by increasing pH, agree in all essentials with those of Lauffer & Price [1940] they need not be given in detail. This effect is not confined to tobacco mosaic virus,

for at 55° both potato virus "X" and tomato bushy stunt virus are rapidly inactivated at pH values that are without appreciable effect at room temperature.

Table 1. *The effect of sodium dodecyl sulphate on tobacco mosaic virus*

Sample	Concentration of S.D.S.	Serological titre	Average no. of lesions per leaf at	
			10 ⁻⁴	10 ⁻⁵
A	0	1/1,280,000	6	1
B	0.043 %	1/640,000	2	1
C	0.073 %	1/160,000	0	0
D	0.143 %	1/10,000	0	0
E	0.073 %	1/1,280,000	104	47
	neutralized			
F	0 control	1/2,560,000	205	95

Samples A, B, C, and D were 0.3 % solutions of tobacco mosaic virus in *M*/15 glycine: NaCl buffer at pH 9.3 exposed for 1 hr. at 55° to the concentration of S.D.S. stated. They were then taken to pH 6 with HCl and phosphate buffer and diluted for testing. In E the virus was added to a neutralized and diluted buffer mixture containing as much S.D.S. as C, and in F virus was added to neutralized buffer only.

Table 1 shows the effect of S.D.S. on tobacco mosaic virus at pH 9.3 and 55°. In the absence of S.D.S. there is a definite fall in the infectivity of the preparation that is not paralleled by a similar fall in serological titre. A similar dissociation of these two properties with tobacco mosaic virus can be produced by oxidizing agents, nitrous acid, formaldehyde and irradiation with X-rays or ultra-violet [Bawden & Pirie, 1937; Stanley, 1936], and with tomato bushy stunt virus by alkali [Bawden & Pirie, 1938, 2]. The conditions of pH and temperature within which there is complete, or almost complete, loss of infectivity with tobacco mosaic virus without great loss of serological activity are narrowly circumscribed, and it is seldom that such a definite difference as that between samples A and E in Table 1 is obtained. Samples which lose their infectivity but not their serological activity in this manner retain their ability to show anisotropy of flow. With slightly more severe treatment, however, secondary changes rapidly follow those that lead only to a loss of infectivity, and the preparations lose both their serological activity and anisotropy of flow.

It is clear that the action of alkali on tobacco mosaic virus is complex. Eriksson-Quensel & Svedberg [1936] and Wyckoff [1937] found that after exposure to alkali between pH 9 and 11 tobacco mosaic virus was split into a number of products of smaller molecular weight and that the extent of the disintegration depended on the pH. These workers made no measurements on the activities of their preparations after these treatments, but Best [1936] found that both purified and crude preparations were 50 % inactivated by 12 hr. exposure to pH 8.2 at 17°. The purified preparations that we have used have been much more resistant to alkali than this. Table 2 shows the results of one of our experiments. There was no difference between the control and a sample that had been held at pH 8.58 for 24 hr. at 20°, and only above pH 10 was there great inactivation. The treatment at pH 10.5 again shows the dissociation of infectivity from serological activity. The treatment at pH 9.3 shows an effect which we have frequently obtained in these alkali tests, an apparent increase in the infectivity by gentle treatment. This activation can be regarded as a partial reversal of the fall in infectivity that is produced by rigorous chemical purification of the virus. This is believed to be caused by the linear aggregation of

Table 2. *The effect of alkali on tobacco mosaic virus*

pH	Time	Serological titre	Average no. of lesions per leaf at	
			10 ⁻⁴	10 ⁻⁵
11	24 hr.	1/40,000	0	0
10.5	24 hr.	1/3,000,000	12	2
9.3	24 hr.	1/4,000,000	106	21
8.5	24 hr.	1/4,000,000	74	9
8.5	5 min.	1/4,000,000	65	11
	Control	1/4,000,000	73	10

In each sample 0.1 ml. of 5.3% tobacco mosaic virus solution was added to 1 ml. of *M*/10 glycine: NaCl buffer at the pH stated. 0.08 and 0.06 ml. of *N*/10 NaOH were added to the first and second, for interpolation on the titration curve of tobacco mosaic virus shows that these amounts are necessary to bring 5.3 mg. of virus to pH 11 and pH 10.5 respectively. After the time stated, each was neutralized with acetic acid and diluted for testing. In the control, virus was added to 1 ml. of pH 8.5 buffer previously neutralized with acetic acid.

virus particles, and the simplest explanation of the reactivation is that the alkali disaggregates the long rods. Thus at least three successive effects of alkali on tobacco mosaic virus can be detected. First, there is a disaggregation of the purified material, secondly, a change within the particles that renders them non-infective without destroying their structure or serological properties and thirdly, a disruption of the particles that leads to a loss of all characteristic properties. The course of the third stage is also influenced by the temperature, for at low temperatures the products of denaturation remain soluble, whereas at high temperatures they coagulate.

The action of S.D.S. in the presence of alkali differs from the action of alkali alone. For example, in the presence of sufficient S.D.S. to cause appreciable inactivation of tobacco mosaic virus there is no precipitate of denatured protein such as would be produced by the action of alkali and temperature alone. Thus, in the treatments described in Table 1, although A contained a precipitate, B and C were only opalescent and D was perfectly clear. In attempts to gain further information on the antigenic constitution of tobacco mosaic virus, rabbits were injected with the phosphorus-free protein obtained by treating the virus with S.D.S., but the serum failed to react with either intact virus or that treated with S.D.S.

Tomato bushy stunt virus is even less readily attacked by S.D.S. than tobacco mosaic virus, and in neutral solution it has never been completely inactivated in any of our experiments. This may be because an equilibrium is established, but we have no definite information on this. In solutions containing 4.4% S.D.S. and 2.6% bushy stunt virus and 3.5% Na₂HPO₄·12H₂O precipitates separate almost immediately, the precipitates appearing amorphous under the microscope. Like the similar, though shimmering, precipitates that separate with tobacco mosaic virus, these dissolve in about 30 min. at 37°. In these conditions a second precipitate, apparently a complex of S.D.S. and modified protein, separates after a few hours; this precipitate, like that given by the unchanged virus with ammonium sulphate, dissolves when the fluid is cooled. After 20 hr. at 37°, a dialysed sample still contains about 10% of unchanged virus. This mixture can be partially separated either by centrifuging at 16,000 r.p.m. or by ammonium sulphate precipitation, for the inactive products formed by the action are not sedimented by a few hours' centrifuging at this speed and they are more easily precipitated by ammonium sulphate than the virus, especially at 0°. The inactive

protein that can be separated by the addition of ammonium sulphate to slightly alkaline solutions is almost free from phosphorus and carbohydrate, and nucleic acid can be precipitated from the supernatant fluids by the addition of acid.

With tomato bushy stunt virus it is much easier to destroy infectivity without destroying serological activity by means of alkali than it is with tobacco mosaic virus. We have previously described [Bawden & Pirie, 1938, 2] this effect when bushy stunt virus is exposed to *pH* 11 at 18°, but at higher temperatures the effect is more definite and can be produced in less alkaline solutions. In one experiment exposure for 30 min. at 55° at *pH* 9 and 9·7 in *M*/10 glycine buffer gave no change in the serological titre, although at a dilution of 10⁻⁴ g. of protein per ml. the samples gave 3 and 0 lesions respectively, whereas the control mixed with neutralized buffer at 55° gave 720 lesions. From preparations treated in this manner rhombic dodecahedral crystals can be obtained by slow precipitation with ammonium sulphate that are apparently identical with those formed by fully active virus. This non-infective crystalline material is indistinguishable, by the chemical, physical and serological tests that we have applied, from the virus isolated directly from infective sap, but careful solubility measurements have not yet been made. The existence of a protein that is not infective although it closely resembles the active virus in its properties suggests the possibility that such a material may often be a contaminant of purified virus preparations as normally prepared. This is especially likely as a similar type of inactivation seems to proceed in crude infective sap, although here, as the *pH* is usually around 5·5, the effect cannot be attributed to alkaline inactivation. Smith [1937] observed that infective sap from bushy stunt plants rapidly lost its infectivity and was inactive within a month. We have confirmed this observation but have found in striking contrast that purified preparations do not lose their infectivity in this manner. After more than a year at room temperature the infectivity of purified preparations of this virus has not been found to be significantly reduced. Samples of infective sap have been tested for serological activity and infectivity over periods of time, and although in all the infectivity has been found to fall off rapidly the serological activity has remained constant. Samples of infective sap kept over a year at room temperature and which had been non-infective for a year were still found to be serologically active, and from them we isolated a non-infective crystalline nucleoprotein by the methods used for normal virus isolation. This material was found in the same quantities and gave the same serological titre as fully active virus preparations. The possibility that preparations may consist of mixtures of infective and non-infective nucleoprotein may explain the fact that different preparations give remarkably constant serological titres but differ in their infectivities.

Bushy stunt virus preparations lose their activity in alkaline solutions more rapidly in the presence of S.D.S. and inactivation occurs at lower *pH* values. The action is also not restricted to one that merely destroys infectivity, for the nucleic acid is split off from the non-infective protein and the preparation loses its serological activity. In one test in which a 1% solution of the virus was exposed for 160 min. to 2·6% S.D.S. at *pH* 9 and 18°, the preparation lost its infectivity but could be split into various fractions after dialysing for a week at 0°. The addition of 1/8 vol. of saturated ammonium sulphate solution precipitated a nucleic acid-free protein, which represented 75% of the original weight of virus, was amorphous and did not react with virus antiserum. With more ammonium sulphate there was slow crystallization at 0° of a non-infective, though serologically active, nucleoprotein similar to that already described. The yield of this was 12% of the starting material.

The absorption spectrum of tomato bushy stunt virus has a pronounced maximum at 260 $m\mu$. After partial inactivation by S.D.S. the curve has the same shape but it lies below the curve for fully active virus. Thus a 2 cm. layer of a 0.016% solution of the virus transmitted 1/100 of the incident light at 260 $m\mu$ ($\log_{10} I_0/I = 2$), whereas after treatment with S.D.S. so that half of the virus was destroyed the transmission was doubled at 260 $m\mu$ ($\log_{10} I_0/I = 1.7$). We have described a similar result during the inactivation of potato virus "X" and tobacco mosaic virus by urea, during which the nucleic acids are separated from the proteins. In the inactivation of bushy stunt virus by urea, on the other hand, the nucleic acid is not liberated, and there is no such decrease in opacity.

Urethane

The pharmacological action of the urethanes led naturally to their use in the study of enzyme systems, and Warburg & Wiesel [1912] pointed out that there was a correlation between the ability of a narcotic to give a precipitate with yeast maceration juice and its inhibitory action on the respiration of various tissues. Meyerhof [1918] confirmed these observations, and found that the presence of salt was necessary for the formation of a precipitate by urethanes in protein solutions. Hopkins [1930] measured the rate of denaturation of egg albumin in urethane and the slower rate of denaturation of sheep serum proteins, and he commented on the fact that the solvent powers of urethane were less than those of urea. On the other hand, Jirgensons [1936] found that 25% methylurethane impeded the precipitation of casein by salts.

In neutral solution strong ethylurethane rapidly denatures tobacco mosaic virus and is a solvent for the denatured products even in the presence of salts. At pH 7 the anisotropy of flow of virus solutions disappears after a few minutes in 28–40% solutions of urethane and the solutions become perfectly clear, although on dilution a precipitate develops if traces of salt are present. With more dilute urethane the anisotropy of flow does not quite disappear, the loss of infectivity and serological activity is only partial and the inactivated virus precipitates although it can readily be dissolved by the addition of more urethane. The rate of inactivation of tobacco mosaic virus by urea is greatly increased by cooling below 20°, but inactivation by urethane is not. After 48 hr. at 0° a 1.5 *M* solution of urethane in *M*/30 phosphate buffer of pH 7 containing 0.6 g. of virus per litre gave a serological titre of 1/640,000 and an average number of lesions per leaf of 68 and 5 at dilutions of 10^{-4} and 10^{-5} respectively. After the same time at 0° the preparation gave the same titre and the lesions were 45 and 5, whereas the control gave a titre of 1/1,280,000 and 86 and 19 lesions per leaf.

Table 3. *The effect of urethane on potato virus "X"*

Concentration of urethane <i>M</i>	Serological titre	Average no. of lesions per leaf at	
		10^{-4}	10^{-5}
0.5	1/1,280,000	171	52
1.0	1/640,000	137	41
2.26	No ppt. at 1/10,000	0	0
Control	1/1,280,000	193	64

In each sample a 0.08% solution of potato virus "X" in *M*/50 pH 7 phosphate buffer was exposed for 24 hr. at 18° to the concentration of urethane stated. They were then diluted with 3 vol. of water and diluted further for testing after 36 hr. For the control an amount of urethane equal to that in the *M* sample was added immediately before diluting and testing.

The solubility of urethane at 0° is too small to permit experiments under conditions in which inactivation is more complete.

Tomato bushy stunt virus is much more resistant to urethane than is tobacco mosaic virus; 22 hr. exposure at 23° altered neither the serological titre nor the infectivity of a 0.14% virus solution in 0.023*M* phosphate buffer at pH 7.8. Potato virus "X", however, is more susceptible and Table 3 shows the inactivation of this virus by urethane. The denatured virus was not held in solution by 2.26*M* urethane and at that concentration the anisotropy of flow disappeared almost immediately and a precipitate separated. At the lower concentrations there was no precipitation and only a reduction in the anisotropy of flow

Guanidine

Guanidine, like many of the other agents used in these experiments, affects proteins in a number of different ways. Petrumkin & Petrumkin [1927; 1928] found that gelatin and a mixture of denatured proteins from brain combined with guanidine in alkaline solution, and Grynberg [1933] measured the amount of combination under various conditions of pH, salt and guanidine concentration, with casein, gelatin, egg albumin and a globulin. Svedberg [1937] studied the dispersive action of guanidine on haemocyanin by means of the ultracentrifuge, and its action on myosin was studied by Edsall & Muhl [1940] who followed the loss of anisotropy of flow and viscosity. Denaturation with the production of —SH groups was found with egg albumin [Greenstein, 1938], excelsin, edestin and globin [Greenstein, 1939], tobacco mosaic virus [Stanley & Lauffer, 1939] and with myosin [Greenstein & Edsall, 1940]. Where comparative tests have been made guanidine has been found to denature at lower concentrations than other agents such as urea, and to produce a larger number of —SH groups. Greenstein [1938; 1939] found with both urea and guanidine that doubling the concentration, within the critical range, more than doubles the amount of —SH produced. This result parallels the well-known effects of variations in the concentration of disinfectants and agrees with the effects of variations in the concentration of the various virus-inactivating agents described in this paper.

Neutral solutions of guanidine hydrochloride, if more concentrated than 0.2*M*, precipitate tobacco mosaic virus from solution. The precipitate can be washed with guanidine solution, but readily dissolves in water. Like the precipitate produced when clupein is added [Bawden & Pirie, 1937], it has a fibrous structure, but unlike the clupein precipitate it is not dissolved by the addition of small amounts of salt. There is no loss of activity during this precipitation provided that solutions less than *M* are used, but in more concentrated solutions there is some denaturation and then the precipitate produced is amorphous. In guanidine solutions more concentrated than 2.5*M* the active virus does not precipitate, but there is rapid inactivation and a precipitate of denatured protein separates. The course of inactivation of tobacco mosaic virus by strong guanidine and its precipitation by dilute guanidine are illustrated in Table 4.

Tomato bushy stunt virus differs from tobacco mosaic virus in its behaviour towards guanidine. It does not precipitate with dilute solutions and concentrated solutions retain the denatured virus in solution. For example, virus exposed to 3.3*M* guanidine did not precipitate on dilution. After inactivation by exposure for 21 hr. at 23° to 1.3*M* guanidine, however, a precipitate separated on dilution. It is clear that with these two viruses, as with the proteins that Greenstein has studied, guanidine is effective at lower concentrations than urea.

Table 4. *The effect of guanidine on tobacco mosaic virus*

Treatment	Serological titre	Average no. of lesions per leaf at	
		10 ⁻⁴	10 ⁻⁵
A. 24 hr. in 2.7 <i>M</i> guanidine hydrochloride	No ppt. at 1/10,000	0	0
B. 24 hr. in 2.25 <i>M</i> guanidine hydrochloride	1/80,000	36	4
C. 5 min. in 0.68 <i>M</i> guanidine hydrochloride, then centrifuged:			
(1) Precipitate	1/640,000	200	80
(2) Supernatant	No ppt. at 1/10,000	2	0
D. Control; guanidine hydrochloride added after dilution	1/640,000	209	85
E. Control; no guanidine hydrochloride	1/640,000	228	99

All the tests were made at 17° with 1 ml. samples of a 1.1% solution of tobacco mosaic virus in 0.075 *M* phosphate buffer at pH 7. To A and B were added 0.4 and 0.2 ml. of 3.38 *M* guanidine hydrochloride, to C 0.3 ml. of water and 0.2 ml. of guanidine, and to D 2.2 ml. of water and 0.2 ml. of guanidine. After the stated time each was made up to 2.5 ml. with water, and diluted further for testing 36 hr. later.

Pyridine, picoline, lutidine, aniline and nicotine

The action of aqueous pyridine on proteins has been but little studied, although it has long been known to dissolve some [Levites, 1911] and to make gelatin swell even when neutralized [Fischer & Sykes, 1915]. Spiro [1900] observed that dilute pyridine lowered the temperature of coagulation of proteins and changes in haemoglobin [Jirgensons, 1936] and tobacco mosaic virus [Bawden & Pirie, 1937] have been brought about by it.

The neutral pyridine used in our tests was made by diluting 8 ml. of pyridine and 0.5 ml. of *N*-HCl to 25 ml. with water. Neutral picoline was made in the same way. In 3 *M* pyridine tobacco mosaic virus and potato virus "X" lose their anisotropy of flow almost immediately and give clear solutions from which small, sticky precipitates separate after 24 hr. In more concentrated pyridine the inactivated viruses do not precipitate, while in more dilute pyridine the anisotropy of flow disappears slowly and there is an increase in opalescence leading finally to precipitation. The behaviours of these two viruses in picoline are similar to those in pyridine, but picoline has a slightly greater inactivating power. Tables 5 and 6 illustrate the inactivating effects of pyridine and picoline on tobacco mosaic virus. Potato virus "X" is inactivated under much the same conditions as tobacco mosaic virus, but tomato bushy stunt virus is more resistant. Thus, 20 hr. exposure at 18° to either 2.4 *M* pyridine or 1.3 *M* picoline

Table 5. *The effect of pyridine on tobacco mosaic virus*

Concentration of pyridine <i>M</i>	Serological titre	Average no. of lesions per leaf at	
		10 ⁻⁴	10 ⁻⁵
1.33	No ppt. at 1/10,000	0	0
0.625	1/640,000	101	54
Control	1/640,000	118	72

Tobacco mosaic virus solutions containing 0.052% virus and *M*/30 pH 6.95 phosphate buffer were exposed to the concentration of pyridine stated for 24 hr. at 16°. They were then diluted so that the pyridine was 0.312 *M* and were further diluted for testing 36 hr. later. In the control 0.312 *M* pyridine was added to the virus immediately before diluting and testing.

Table 6. *The effect of picoline on tobacco mosaic virus*

Concentration of picoline <i>M</i>	Serological titre	Average no. of lesions per leaf at	
		10 ⁻⁴	10 ⁻⁵
0.6	1/80,000	12	3
0.4	1/640,000	66	21
0.2	1/1,280,000	104	51
Control	1/2,560,000	165	69

Each sample contained 1.2 mg. of tobacco mosaic virus and 0.1 ml. of *M*/10 pH 7 phosphate: borate buffer and 0.3 ml. of *M* picoline in a total volume of either 0.5, 0.75, 1.5 or 2.5 ml. The first three samples were kept 18 hr. at 18° when they were diluted to 2.5 ml., and after 36 hr. they were further diluted for testing. The picoline in the control was added immediately before diluting and testing.

had no effect on the serological titre of bushy stunt virus and only lowered the infectivity slightly. A commercial mixture of lutidines was also tested on tobacco mosaic virus. This was as good a precipitant as pyridine or picoline, i.e. precipitation was complete after 1 hr. in a 4% solution, but its solubility in water was insufficient to give concentrations great enough to have any solvent action on the denatured virus. Picoline, lutidine and pyridine all separate the nucleic acid from the protein in tobacco mosaic virus.

The protein-precipitating power of aniline has often been commented upon [e.g. Spiro, 1900], and it is therefore surprising that Lauffer [1938] found that exposure for 10 min. to concentrated mixtures of aniline and glycerol had no effect on the infectivity of tobacco mosaic virus. We have been unable to confirm Lauffer's result; 2 vol. of a 2.35% solution of purified tobacco mosaic virus were added to 98 vol. of a mixture of 48 parts of glycerol and 50 parts of aniline (one of the mixtures used by Lauffer), and samples were withdrawn and diluted for infectivity tests. Dilutions of the virus in water and in the already diluted aniline: glycerol mixture were used as controls. Table 7 shows the results from

Table 7. *The effect of a glycerol: aniline mixture on tobacco mosaic virus*

Time of contact	Average no. of lesions per leaf at	
	10 ⁻⁵	10 ⁻⁶
Diluted immediately	18	10
30 min.	10	6
90 min.	7	4
4 hr.	10	6
Virus added to diluted mixture	83	20
Virus diluted in buffer	84	19

Description in text.

one such experiment. It is apparent that contact of the virus with the glycerol: aniline mixture caused a great immediate reduction in infectivity, but the reduction did not increase greatly as the time of contact was extended. That the effect was one of the mixture on the host plant seems improbable, for at the dilution present in the inoculum there was no fall in infectivity. There is no obvious reason for the difference between Lauffer's results and our own, but the possibility that different strains of tobacco mosaic virus may differ in their resistances to inactivation must be remembered.

The birefringence of tobacco mosaic virus in neutral, salt-free solutions containing up to 20% of nicotine remains unaffected even after several days and

no precipitate separates. In the presence of salt, however, an opaque, fibrous precipitate separates after a few hours. On gentle shaking this precipitate disappears completely, leaving a fluid indistinguishable in appearance from a normal virus preparation. After a few hours the precipitate again appears and can again be dispersed by shaking; the process can apparently be repeated indefinitely over a period of weeks. A suitable mixture for demonstrating this phenomenon contains 0.4 % of virus, 4 % of NaCl and 7 % of nicotine neutralized with acetic acid, but other mixtures, e.g. those made from neutralized nicotine tartrate, work equally well. Similar, though rather less striking, results are obtained by dissolving tobacco mosaic virus in neutral *M* arginine hydrochloride. Exposure to this type of precipitating agent does not inactivate the virus, and 2 months' exposure to 15 % nicotine at pH 7 and 18° had no appreciable effect on the infectivity and serological activity. Fukushi [1930] found that 3 % nicotine caused partial inactivation of tobacco mosaic virus in 3 days, but as he was using crude infective sap there is no necessary contradiction between his results and ours.

There are two analogies for this type of reversible precipitation. The first is the birefringent liquid layer that separates from concentrated virus preparations [Bawden & Pirie, 1937], for by vigorous shaking this can be resuspended in the form of tactoids that are too small to be seen and have a refractive index too close to that of the surrounding medium to give a visible opacity. Best [1937] described a precipitate settling from an old sample of clarified, infective sap that may well be identical with the nicotine precipitate. This was also dispersed on shaking and reformed on standing undisturbed. In addition to the physico-chemical interest attached to precipitates of this type, their formation, especially in fluids with compositions similar to plant sap, may throw some light on the mechanism of formation of the characteristic inclusion bodies found in plants infected with certain viruses. The other systems that have been considered as models for this phenomenon [Bawden & Sheffield, 1939] bear less resemblance to normal cell contents than those described here.

Phenol, salicylic acid and benzoic acid

Runge [1834], the discoverer of phenol, found that it was a protein coagulant, and its power of inhibiting enzymes was stressed by Plugge [1872], who was also one of the first to use it as a disinfectant. Buchner & Hoffmann [1907] and Duchacck [1909] correlated this inhibitory power with the precipitation of protein. Innumerable studies with bacteria and animal viruses have shown that 5–10 % phenol is usually lethal. Henderson [1933] found that the stability of tobacco ringspot virus in crude sap was increased by the addition of 0.25 % phenol, and Stanley [1935] found that 1 % phenol had little effect on tobacco mosaic virus. Bawden [1935] found that 2 % phenol acting for 48 hr. at 1° had no effect on potato virus "X", whereas 3 % destroyed infectivity and either greatly reduced or destroyed serological activity.

In concentrated solutions phenol is usually a protein solvent, but the molecular weights found for proteins dissolved in this manner have been so low [Troensgaard & Schmidt, 1924; Cohn & Conant, 1926] that it is probable that they were dissociated. Measurements in the ultracentrifuge appear to confirm this dissociation [Lundgren, 1938]. The effects of salicylates appear to be similar to those of phenol. Dilute solutions precipitate proteins [von Furth, 1896] whereas strong solutions either dissolve proteins [Neuberg, 1916] or cause swelling [Stoeltzner, 1925]. Meissner & Wöhlisch [1937] found that fibrinogen solutions had a maximum opacity when they contained 12.5 % sodium salicylate,

which agrees well with the conclusions of Pauli & Weiss [1931] on the coagulation and solution of a number of proteins. Anson & Mirsky [1933] studied the reversible denaturation of methaemoglobin and found that 0.32 *M* salicylate caused 50 % denaturation. Best [1940] found that dilute salicylate gave a birefringent precipitate with tobacco mosaic virus that was still active, whereas more concentrated solutions gave a precipitate of denatured inactive protein. In 0.5 *M* potassium salicylate a steady state of partial inactivation was set up, although inactivation was almost complete after a few hours at 30° in *M* solution. Tomato spotted wilt virus was much more susceptible, being completely inactivated in 1 hr. at 30° by 0.1 *M* salicylate.

Neuberg [1916] found that most of the aromatic acids had a solvent or "hydrotropic" action on proteins, but the changes that benzoic acid and its derivatives cause when they act on proteins have not been investigated. The swelling action of benzoate on pieces of dura mater was studied by Stoeltzner [1925] and Pauli & Weiss [1931], and Meissner & Wöhlisch [1937] used benzoates and hippurates as protein solvents.

Tables 8 and 9 illustrate the course of inactivation of tobacco mosaic virus and tomato bushy stunt virus by phenol. With these viruses, as with potato virus "X", in the critical range small changes in the phenol concentration have

Table 8. *The effect of phenol on tobacco mosaic virus*

Concentration of phenol <i>M</i>	Serological titre	Average no. of lesions per leaf at	
		10 ⁻⁴	10 ⁻⁵
0.416	No ppt. at 1/10,000	0	0
0.333	1/640,000	42	9
0.25	1/1,280,000	80	21
Control	1/1,280,000	75	22

0.47 % solutions of tobacco mosaic virus were exposed to the concentration of phenol stated for 20 hr. at 23°, when they were diluted for testing. In the control the phenol was added immediately before diluting. The phenol solution was adjusted to pH 7 by the addition of NaOH.

Table 9. *The effect of phenol on tomato bushy stunt virus*

Concentration of phenol <i>M</i>	Serological titre	Average no. of lesions per leaf at	
		10 ⁻⁴	10 ⁻⁵
0.416	1/80,000	0	0
0.2	1/320,000	41	7
Control	1/640,000	74	19

0.16 % solutions of tomato bushy stunt virus at pH 7 were exposed for 21 hr. at 23° to the stated concentrations of phenol, and then diluted and tested.

a large effect on the extent of the inactivation. In the absence of salt phenol does not give a precipitate with either of the viruses, but in the presence of salts a precipitate of denatured protein separates, the extent of the precipitation closely paralleling the loss of activity. As with other inactivating agents such as urea, the precipitate that separates from the inactivated tobacco mosaic virus is free from nucleic acid, whereas that from the inactivated tomato bushy stunt virus still has the nucleic acid attached.

The anisotropy of flow of tobacco mosaic virus preparations is destroyed instantly in 2 *M* sodium salicylate at 20° and in 1.4 *M* it disappears in 10–15 min. These inactive fluids remain quite clear unless they are diluted, when amorphous

precipitates separate. In more dilute salicylate the loss of anisotropy of flow is accompanied by increased opalescence, and in the range M to $0.4M$ there is precipitation of amorphous protein within a few hours. Anisotropy of flow, infectivity and serological activity, although diminished, persist for some days at 18° in $0.4M$ salicylate. As our results with salicylate are in good agreement with Best's [1940], they are not given in detail. Table 10 shows the effects of

Table 10. *The effect of sodium salicylate on tomato bushy stunt virus*

Concentration of salicylate M	Serological titre	Average no. of lesions per leaf at	
		10^{-4}	10^{-5}
1	No ppt. at $1/10,000$	0	0
0.5	$1/320,000$	49	10
0.33	$1/320,000$	66	34
0.2	$1/320,000$	70	27
Control	$1/320,000$	79	37

For the first four samples 0.1 ml. of 1% tomato bushy stunt virus was mixed with 0.1 ml. of neutralized $2M$ sodium salicylate; before the addition of the salicylate, 0.2 ml. of water was added to sample 2, 0.4 ml. to sample 3 and 0.8 ml. to sample 4. After 48 hr. at 23° the samples were diluted for testing. Salicylate was added to the control immediately before dilution and testing.

salicylate on tomato bushy stunt virus. Of the samples only that at M concentration gave any precipitate of denatured protein. In this experiment, as with others, there is evidence that $0.5M$ salicylate may be affecting infectivity without destroying serological activity. At salicylate concentrations $>M$ inactivation occurs rapidly, but the material remains in solution.* Salicylate has a much greater deleterious effect on plant leaves than any of the other agents we have used. Even at dilutions as great as $0.08M$ it is necessary to wash the leaves immediately after inoculation to ensure that they shall not be damaged.

Sodium benzoate at pH 7 inactivates tobacco mosaic virus but only at higher concentrations than those necessary with salicylate; this inactivation is illustrated in Table 11. In the sample exposed to $2M$ benzoate, the anisotropy of flow disappeared in a few minutes and there was precipitation after 15 min., but in the others there was only slight loss of anisotropy of flow and increase in

Table 11. *The effect of sodium benzoate on tobacco mosaic virus*

Concentration of benzoate M	Serological titre	Average no. of lesions per leaf at	
		10^{-4}	10^{-5}
2	No ppt. at $1/10,000$	0	0
1.2	$1/320,000$	64	16
0.8	$1/640,000$	90	25
Control	$1/1,280,000$	105	35
Control, mixed just before testing	$1/1,280,000$	114	37

For each of the three test samples 0.1 ml. of a 2.35% solution of tobacco mosaic virus was added to 0.5, 0.3 and 0.2 ml. of $2.4M$ sodium benzoate solution adjusted to pH 7 with HCl . In addition samples 2 and 3 had had 0.2 and 0.3 ml. of water added. After 20 hr. at 22° they were all diluted to 2.35 ml. with water or benzoate to give a final concentration of $0.5M$ benzoate, and they were further diluted for testing 36 hr. later. In one control virus was added to benzoate at $0.5M$ and tested 36 hr. later; in the other, virus and benzoate were mixed immediately before diluting and testing.

opalescence. Sodium hippurate also inactivates tobacco mosaic virus, but still more slowly; 20 hr. exposure at 23° to 1.9*M* hippurate at *pH* 7 reduced the serological titre from $1/128 \times 10^4$ to $1/32 \times 10^4$, and the average number of lesions per leaf at a virus dilution of 10^{-4} from 105 to 58.

DISCUSSION

Although tomato bushy stunt virus is more susceptible to physical changes such as freezing and drying than either tobacco mosaic virus or potato virus "X", it is more resistant to the inactivating effects of the organic agents used in our experiments. Also, its denaturation often takes a different course, for whereas the commonest effect with the anisotropic viruses is the separation of nucleic acid from the protein, this is unusual with tomato bushy stunt virus. Slight changes within the particle, leading to loss of infectivity without denaturation, are also commoner with bushy stunt virus than with the others.

The number of substances that might profitably have been tested in a survey of the organic agents that inactivate plant viruses is very large, and in this work it was possible to include relatively few. These also have been tested under limited conditions, although our more detailed study of the inactivating effects of urea suggests that many factors such as *pH* or temperature may greatly influence inactivation, and in other conditions it is possible that their actions might be different. Apart from agents that can be regarded as oxidizing agents, acids or alkalis, Spiro, Neuberg, Hopkins, Stanley and Greenstein have, in the papers already quoted, examined the effects of over 100 substances on proteins. These cover a wide chemical range, and the most interesting substances cause irreversible changes in a few hours in neutral solution at room temperature at concentrations below 4*M*. If this limit is imposed, the usual solvents, such as alcohol and acetone, whose precipitating and inactivating actions are well known even though little understood, are excluded except when they are acting on abnormally unstable proteins. Similarly urea, although so widely studied and used as a denaturant, appears to be one of the less active members of the group.

Spiro [1900; 1904], who made the first attempts to systematize the scattered observations of earlier workers, suggested that some of the agents that impede coagulation by heat do so by acting as bases, whereas others, notably the mustard oils, form soluble complexes with proteins. Several authors have adopted the second suggestion, and we have pointed out [Bawden & Pirie, 1940] that a reversible ordered association between urea and proteins is compatible with the observations that have been made on urea: protein systems. Steinhardt [1938] has suggested that if this association occurs at centres normally concerned in the formation of cross linkages between peptide chains in proteins and involves the breaking of the linkage there will, on the removal of the denaturing agent, be a greater tendency for random reformation of cross linkages the larger the number of cross linkages that has been broken. The nature of the cross linkages in proteins is still uncertain, but this picture of their reversible breakage to an extent determined by the concentration of the denaturing agent and with a corresponding change in the probability of their reformation in the original pattern seems to us a reasonably satisfactory interpretation of the observed results. On this picture the efficiency of a denaturing agent depends on its ability to form associations with and to break the normal cross linkages.

At present it is impossible to predict the action of a substance on proteins in solution from a knowledge of its physical and chemical properties, although attempts have been made to correlate the action with changes in surface tension

or dielectric constant. Thus Jirgensons [1936] stressed the significance of the dielectric constant, and showed that the concentration of a substance required to produce maximum opacity in a salt-free colloidal solution was proportional to the dielectric constant. This type of measurement, however, is complicated by the fact that it not only measures the ability of a substance to precipitate or denature a protein but also measures the ability of stronger solutions to act as protein solvents. Steinhardt [1938] concluded that there was no such proportionality, for he found that some active amides raised the dielectric constant whereas others lowered it. Hopkins [1930] drew the following conclusions from his study of denaturation of proteins by nitrogenous substances. "An amide structure is apparently necessary, but in certain relations its activity is lost. Among the ureas, mono-alkyl substitution, or unsymmetrical di-alkyl substitution, leaves the activity qualitatively intact. Symmetrical di-alkyl ureas, on the other hand, are inactive; one amino group must apparently remain unsubstituted. To judge from the case of acetyl urea, however, mono-acetyl substitution removes the activity. In biuret, allantoin and semicarbazide activity is also lost. Acid amides (acetamide and formamide) are active, but all amino-acids tried were without effect and likewise asparagine." However, as there seems to be no good reason to distinguish between the denaturing effects of nitrogenous substances and of non-nitrogenous substances such as benzoate, it is necessary greatly to extend the field in which any useful generalization must be valid. A survey of the literature and our own observations on some 15 substances suggests that the stability of a protein such as tobacco mosaic virus in a 4*M* solution of an organic substance is a more reasonable matter for comment than its instability in such an environment.

SUMMARY

The effects of alkali and of 15 simple organic substances on tobacco mosaic virus and tomato bushy stunt virus are described. Some experiments with potato virus "X" are also included. Bushy stunt virus is the most resistant to denaturation and potato virus "X" the least. The effects of alkali on tobacco mosaic virus are complex; gentle treatment may increase infectivity, slightly more severe treatment causes loss of infectivity but not loss of serological activity, and more severe treatment causes loss of all characteristic properties. With bushy stunt virus inactivation without loss of serological activity occurs over a wider *pH* range, and crystalline non-infective preparations can be made from alkali-treated material. Apparently similar crystalline and non-infective preparations can be isolated from expressed sap allowed to age for some months. In the presence of alkali, sodium dodecyl sulphate readily destroys all the viruses, separating the nucleic acid from the proteins. With the exception of nicotine and arginine, which form with tobacco mosaic reversible, fibrous precipitates, all the substances we have tested at concentrations below 4*M* inactivate the viruses in neutral solution. Dilute solutions of these agents are often precipitants whereas concentrated ones dissolve the products of denaturation. Inactivation of tobacco mosaic virus and potato virus "X" is usually accompanied by the separation of the nucleic acid from the protein, but inactivation of bushy stunt virus is not.

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QUANTITATIVE STUDIES ON THE SEROLOGICAL REACTIONS
OF SOME PLANT VIRUSES AND OF A PEA NODULE BAC-
TERIUM (*RHIZOBIUM LEGUMINOSARUM*).

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IN both agglutination and precipitation, combination between antigen and antibody is followed by the separation of an antigen-antibody complex. The most striking difference between the two reactions is quantitative; in precipitin reactions small amounts of antigen combine with comparatively large amounts of antibody, whereas in agglutination reactions large amounts of antigen react with small amounts of antibody. This difference seems to be controlled by the sizes of the antigens: those giving precipitin reactions are solutions of finely dispersed particles, whereas those giving agglutinin reactions are suspensions of large particles.

The ratio in which a given antigen combines with its antibody in precipitin reactions is a function of the proportions in which the two are mixed (Marrack and Smith, 1931*b*; Heidelberger and Kendall, 1935*a*). However, when the proportions are kept constant at those corresponding to equivalence (i.e. when neither antigen nor antibody is left in the supernatant after the precipitate has been removed by centrifugation), the ratio varies with different sera (Marrack and Smith, 1931*b*). In the serum of one animal it even varies during the progress of immunization, the antibody/antigen ratio becoming larger with each successive immunization (Heidelberger and Kendall, 1935*a*). In addition

to these variations in the combining proportions of any given antigen with its antibody, the antibody/antigen ratio at equivalence point varies widely with antigens of different sizes, decreasing with increasing size.

Most previous work has been done with relatively small antigens, the largest protein molecules investigated being haemocyanins. Purified preparations of some of the more stable plant viruses are active antigens, and afford convenient material for quantitative serological investigations on antigens with unusually large particles. In this work investigations were made on tomato bushy stunt virus and its antiserum, and on tobacco mosaic and tomato aucuba mosaic viruses, two serologically related strains, with their homologous and heterologous antisera. For purpose of comparison, the antibody/antigen ratio of still larger particles, namely, a pea nodule bacterium, *Rhizobium leguminosarum*, was also determined.

Tobacco mosaic and aucuba mosaic viruses have rod-shaped particles and give with antisera bulky fluffy precipitates, resembling those given by bacterial flagellar antigens, whereas bushy stunt virus has spherical, or quasi-spherical particles and gives a compact granular precipitate with its antiserum (Bawden and Pirie, 1938). Comparison of the two viruses was therefore of interest to determine whether the shape of the antigenic particles significantly affected quantitative aspects of serological reactions.

MATERIAL AND METHODS.

Solutions of crystallized bushy stunt virus and of tobacco mosaic and aucuba mosaic viruses in the liquid crystalline state, prepared by precipitation methods (Bawden, 1939), were used in all this work.

To prepare the antisera against bushy stunt and tobacco mosaic viruses, rabbits were injected intravenously twice within a week with 2 ml. of 0.2 per cent. virus solutions (sera Nos. 21, 19, 10, 28). One rabbit was injected six times during a period of three weeks with tobacco mosaic virus (serum No. 11). The antiserum against tomato aucuba mosaic virus was prepared by Mr. E. T. C. Spooner in 1937 by a single intravenous injection of 5 mg. of purified virus. The antiserum to the pea nodule bacterium was produced by injecting a rabbit twice a week for three weeks with suspensions made by washing cultures grown on agar slopes with 0.9 per cent. NaCl solution. All the animals were bled from 8 to 10 days after the last injection.

The amounts of precipitate formed by the viruses with their antisera were measured by mixing known weights of virus and known volumes of antiserum, which were then kept for 3 hours at 37° C. and overnight at 1° C. The precipitates were then centrifuged down, washed twice in saline and their nitrogen content determined by micro-Kjeldahl. This was then translated into protein by multiplying by 6.25. The amount of antibody bound by the bacterium was so small that this technique could not be used. Instead, it was estimated by comparing the intensity of colour given with Folin's phenol reagent by the supernatant fluids left after precipitating the bacteria with that given by the antiserum solution. The details are described later.

Constant antibody optimal proportions were found by adding 1 ml. of antiserum at a constant dilution to a series of tubes each containing 1 ml. of

antigen solution at different concentrations. The tubes were immediately placed in a water-bath at 50° C. so that convection currents ensured complete mixing, and the proportions at which precipitation first appeared were taken as optimal. To determine constant antigen optimal proportions 1 ml. of antigen solution at a constant dilution was added to 1 ml. of antiserum at various dilutions. The equivalence point with the virus antigens corresponded with the constant antibody optimal proportions. On the other hand, with pea nodule bacteria constant antigen optimal proportion corresponded with the minimum amount of antigen required to bind all the antibody.

PRECIPITIN REACTIONS WITH PLANT VIRUSES.

Influence of the Dilution of Reagents on the Amount of Precipitate in the Region of Antigen Excess.

The fact that the volume in which antigen and antibody are dissolved or suspended does not affect appreciably the amount of precipitate formed has been shown for pneumococcal carbohydrate and its precipitin (Heidelberger and Kendall, 1935a), horse pseudoglobulin and its precipitin (Marrack and Smith, 1931a), and pneumococci and their agglutinin (Heidelberger and Kabat, 1937). Experiments were made to find if this was also true for plant viruses and their antisera.

Table I shows the amounts of precipitate formed when bushy stunt virus was mixed with its antiserum in constant proportions but over a range of concentration. In each test the total volume of the fluid was 5 ml. It will be seen that the weight of the precipitate formed is directly proportional to the amounts of antigen and antiserum mixed, and that the ratio of the amount of precipitate to the amount of antigen and antibody is unaffected by the concentration of the reagents over this range of concentration in the region of antigen excess.

TABLE I.—*Amounts of Precipitate Formed by Mixing Increasing Amounts of Bushy Stunt Virus and its Antiserum (No. 10) in a Constant Ratio and Constant Volume.*

Volume of anti-serum used (ml.).		Weight of bushy stunt virus used (mg.).		Weight of precipitate formed (mg.).
0.125	.	0.875	.	0.85
0.25	.	1.75	.	1.7
0.5	.	3.5	.	3.4
1.0	.	7.0	.	6.9

The total volumes were adjusted to 5 ml.
All supernatant fluids showed antigen excess.

Table II shows the results of a similar experiment with tobacco mosaic virus and its homologous antiserum. As with bushy stunt virus, at low antigen concentrations the ratio of the amount of precipitate to the amount of antigen

and antibody is constant. At antigen concentrations greater than 0.2 per cent., however, the increase in the weight of precipitate produced is much greater than the increase in the concentration of antigen and antibody.

TABLE II.—*Amounts of Precipitate Formed by Mixing Increasing Amounts of Tobacco Mosaic Virus and its Homologous Antiserum (No. 21) in a Constant Ratio and Constant Volume.*

Volume of anti-serum used (ml.).	Weight of tobacco mosaic virus used (mg.).	Weight of precipitate formed (mg.).
0.03	2.5	0.62
0.06	5.0	1.3
0.12	10.0	3.1
0.25	20.0	13.3

The total volumes were adjusted to 5 ml.
All supernatant fluids showed antigen excess.

As will be seen later, there is close agreement between the amounts of precipitate obtained with 0.01 ml. of serum No. 11 to which varying amounts of tobacco mosaic virus were added and those obtained with different volumes of serum No. 11 and different amounts of tobacco mosaic virus, the total volume being always adjusted to 5 ml. (Table VI). This also shows that the amounts of precipitate formed by tobacco mosaic virus and its antiserum do not depend appreciably upon the concentration of both reagents over a range of antibody/antigen ratios, being merely a function of their quantities.

Effect of Varying Proportions of Antibody and Antigen.

Bushy stunt virus and its antiserum.

Table III shows the amounts of precipitate obtained when bushy stunt virus was mixed with its antiserum (No. 28) in proportions varying from anti-

TABLE III.—*Amounts of Precipitate Formed by Bushy Stunt Virus with its Antiserum No. 28 Mixed in Varying Proportions.*

Volume of anti-serum used (ml.).	Weight of antigen used (mg.).	Weight of precipitate formed (mg.).	Tests of supernatant fluids with—	
			Antiserum.	Antigen.
4.0	0.2	0.48
4.0	0.4	0.96
1.0	0.25	0.5	0	+++
1.0	0.5	0.9	0	+++
1.0	1.0	1.5	0	++
1.0	2.0	2.6	0	+
1.0	3.0	3.7	0	±
1.0	5.0	4.9	+	0
1.0	7.0	5.8	+++	0
0.25	2.0	1.4
0.12	2.0	0.5
0.06	2.0	0

The total volumes adjusted to 5 ml.

body excess to antigen excess. As the concentrations of bushy stunt virus in all the tests fall within the range shown in Table I, in which varying concentration has no effect on the ratio of the weight of precipitate to the amount of antigen and antibody, provided the two are in constant proportions, it seems to be justifiable to translate these results into the weights of precipitate that would be obtained by adding varying amounts of antigen to 1 ml. of antiserum. This has been done, and the result is shown in Fig. 1, where the weight of antigen added to 1 ml. of antiserum No. 28 is plotted against the weight of precipitate produced.

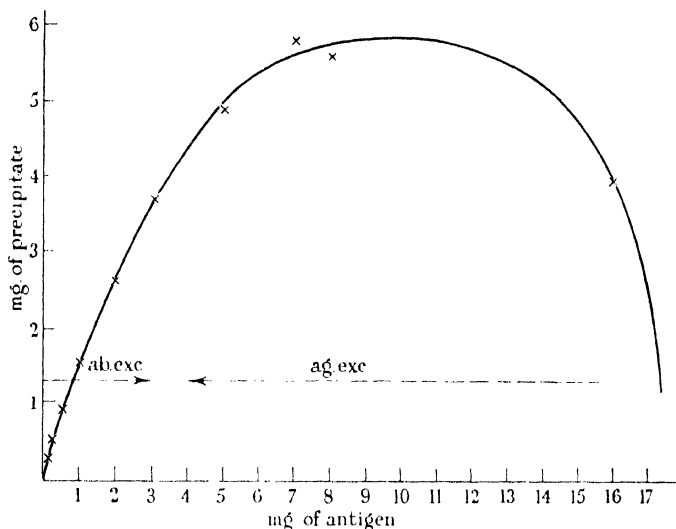


FIG. 1.—Amounts of precipitate formed by 1 ml. of bushy stunt virus antiserum (No. 28) with varying amounts of bushy stunt virus. *ab. exc.* antibody excess; *ag. exc.* antigen excess.

The equivalence point is when 3 mg. of virus is added to 1 ml. of the antiserum, and as at this point 3.7 mg. of precipitate are formed, it is reasonable to assume that 1 ml. of serum No. 28 contains approximately 0.7 mg. of antibody. The weight ratio antibody/antigen at equivalence is thus about 0.25. The maximum precipitate is obtained in the antigen excess zone, when the amount of antigen added is about twice that needed for equivalence. It is apparent that when more than about 17 mg. of virus are added to 1 ml. of this serum no precipitate is formed, i.e. any antibody/antigen complex formed in these conditions is soluble. If the amount of antibody is taken as 0.7 mg., its molecular weight as 160,000 (Heidelberger and Pedersen, 1937; Kabat and Pedersen, 1938) and the molecular weight of bushy stunt virus as 8,000,000 (McFarlane and Kekwick, 1938), then the ratio 17 mg. virus/0.7 mg. of antibody corresponds to one molecule (or particle) of virus to two molecules of antibody. When the ratio of antibody to antigen is increased slightly above this a precipitate separates. If therefore it can be assumed that in the region

of antigen excess the antibody available is equally distributed on all the antigen particles, it is apparent that at least three antibody molecules must combine with one virus particle to render it insoluble. At the equivalence point 3 mg. of virus combine with 0.7 mg. of antibody, and making the same assumptions about molecular weights, one virus particle combines with about 12 molecules of antibody. The combining proportions of antibody and antigen vary somewhat with different sera, and in similar experiments with serum No. 10 (a somewhat stronger antiserum) the weight ratio of antibody/antigen at equivalence was found to be 0.35, instead of 0.25, and the molecular ratio to be 18 of antibody to 1 of virus instead of 12.

Aucuba and tobacco mosaic viruses and tobacco mosaic virus antiserum.

Table IV shows the results of a similar experiment on the precipitation of aucuba and tobacco mosaic viruses with tobacco mosaic virus antiserum (No.

TABLE IV.—*Amounts of Precipitate Formed by Tobacco Mosaic and Aucuba Mosaic Viruses with Tobacco Mosaic Virus Antiserum (No. 11).*

Volume of antiserum used (ml.).	Weight of antigen used (mg.).	Weight of precipitate formed (mg.).	Tests of supernatant fluids with—			
			Anti-tobacco mosaic serum.	Anti-aucuba serum.	Tobacco mosaic virus.	Aucuba mosaic virus.
4.0	0.2	0.88
4.0	0.4	1.6
1.0	0.25	0.7	0	..	+++	..
1.0	0.5	1.0	0	..	++	..
1.0	1.0	1.5	0	..	++	..
1.0	2.0	2.5	0	..	+	..
1.0	3.0	3.6	0	..	0	..
1.0	4.0	4.1	+	..	0	..
1.0	8.0	6.0	++	..	0	..
0.1	2.0	1.5
0.05	2.0	1.2
0.025	2.0	0.8
0.014	2.0	0.4
0.012	2.0	0.1
0.006	2.0	0

<i>Aucuba Mosaic Virus.</i>						
1.0	0.5	0.96	0	0	++	+
1.0	1.0	1.6	0	0	0	0
1.0	2.0	2.6	±	±	0	0
1.0	4.0	3.9	++	++	0	0
1.0	8.0	6.2	+++	+++	0	0
0.05	2.0	1.4
0.025	2.0	0.9
0.012	2.0	0

The total volumes adjusted to 5 ml.

11) when mixed in different proportions. Here again as all the concentrations of the viruses used fall within the range in which the weight of precipitate is directly proportional to the amounts of antigen and antibody mixed, the results in Table IV can be computed for the weight of precipitate produced by 1 ml. of antiserum. This has been done, and the results plotted are shown in Fig. 2.

At the equivalence point, when 3 mg. of tobacco mosaic virus is added to 1 ml. of antiserum, 3.6 mg. precipitate are formed; the combining proportions by weight of antibody and antigen are 0.2, and the amount of antibody per

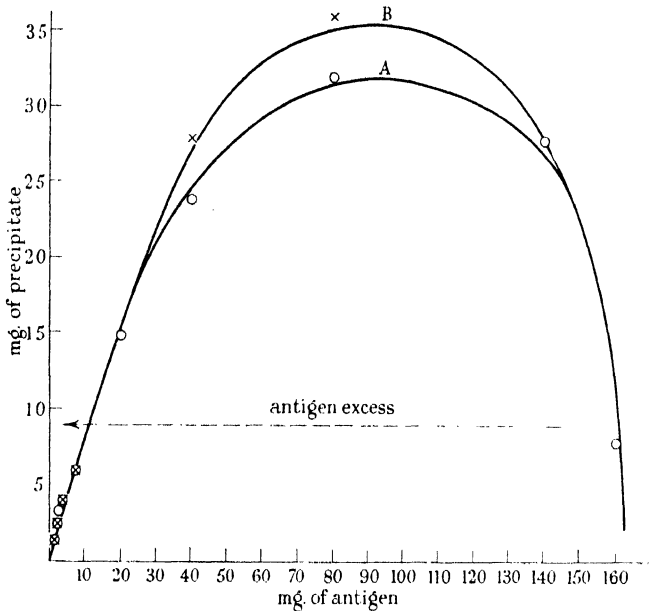


FIG. 2.—Amounts of precipitate formed by 1 ml. of tobacco mosaic virus antiserum (No. 11) with varying amounts of tobacco mosaic virus (curve A) and aucuba mosaic virus (curve B).

ml. of serum is approximately 0.6 mg. Provided the antigen concentration does not exceed 0.2 per cent., the addition of 160 mg. or more of tobacco mosaic virus to 1 ml. of antiserum does not give any precipitate. The exact weight of tobacco mosaic virus particles is not known, and probably varies, but if the figure of 50,000,000 times the weight of a hydrogen atom given by Kausche and Ruska (1939) from measurements in the electron microscope is taken, then 160 mg. of virus and 0.6 mg. of antibody correspond to a ratio of one virus particle to one antibody molecule. If the ratio of antibody/antigen is increased slightly above this a precipitate separates. Thus, if again it can be assumed that the available antibody is distributed equally over the antigen, a complex formed by one virus particle and one antibody molecule is soluble, but combination with more than one antibody molecule is sufficient to render the

antibody-antigen complex insoluble. Making the same assumptions about molecular and particular weights, at the equivalence point one virus particle combines with 70 antibody molecules. Similar combining proportions were found for a second serum, No. 21, which was slightly weaker and contained 0.4 mg. of antibody per ml.

The greatest amount of precipitate for a given amount of antibody is obtained much further in the region of antigen excess with tobacco mosaic virus than with bushy stunt virus, it being obtained when about 30 times the amount of antigen required for equivalence is added, instead of twice. Similarly, the maximum amount of precipitate obtained is also much greater with tobacco mosaic virus, it being about 10 times that obtained at the equivalence point instead of less than twice as with bushy stunt virus.

The behaviour of aucuba mosaic virus with tobacco mosaic virus antiserum No. 11 was almost identical with that of tobacco mosaic virus itself, and except for the fact that it actually produced a little more precipitate in

TABLE V.—*Amounts of Precipitate Formed by Aucuba Mosaic and Tobacco Mosaic Viruses with Aucuba Mosaic Virus Antiserum (No. 392).*

				<i>Aucuba Mosaic Virus.</i>			
Volume of antiserum used (ml.).	Weight of antigen used (mg.).	Weight of precipitate formed (mg.).		Tests of supernatant fluids with—			
				Anti-aucuba serum.	Anti-tobacco mosaic serum.	Aucuba mosaic virus.	Tobacco mosaic virus.
0.5	0.25	0.66	.	++++	..	0	..
0.5	0.5	0.9	.	+	..	0	..
0.5	1.0	1.66	.	0	..	0	..
0.5	2.0	2.72	.	0	..	0	..
0.5	4.0	4.6	.	0	..	+	..
0.1	2.0	2.1
0.05	2.0	1.6
0.033	2.0	1.2
0.025	2.0	0.7
0.022	2.0	0.6
0.012	2.0	0
<i>Tobacco Mosaic Virus.</i>							
0.5	0.25	0.4	.	0	0	++	±
0.5	0.5	0.7	.	0	0	+	0
0.5	1.0	1.2	.	±	+	+	0
0.5	2.0	1.95	.	+	+	+	0
0.5	4.0	3.8	.	+++	+++	+	0
0.1	2.0	1.5
0.05	2.0	0.7
0.033	2.0	0.5
0.025	2.0	0

The total volumes adjusted to 5 ml.

the region of antigen excess the curves for the two viruses could almost be superimposed (Fig. 2). The equivalence point was obtained with slightly less aucuba mosaic virus—2 mg. instead of 3 mg. for 1 ml. of antiserum—but the amount of antibody bound by both viruses was the same, namely, 0.6 mg. After removing the precipitate formed at the equivalence point by one virus, the supernatant fluid did not react with the other, showing that all the antibodies produced by tobacco mosaic virus can react with aucuba mosaic virus.

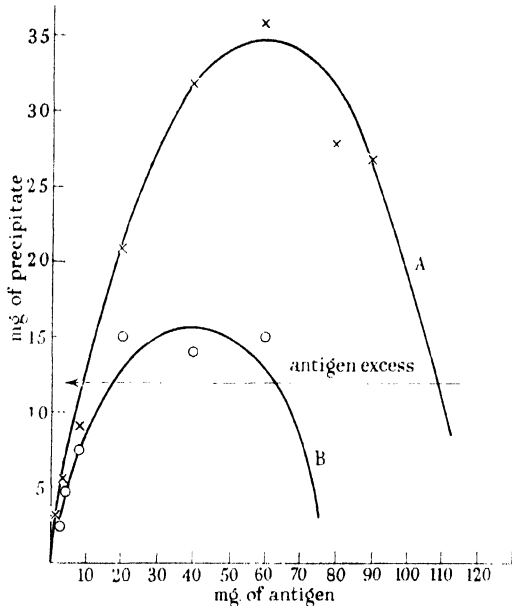


FIG. 3.—Amounts of precipitate formed by 1 ml. of aucuba mosaic virus antiserum (No. 392) with varying amounts of aucuba mosaic virus (curve A) and tobacco mosaic virus (curve B).

Aucuba and tobacco mosaic viruses and aucuba mosaic antiserum.

A different result was obtained when aucuba mosaic and tobacco mosaic viruses were tested against aucuba mosaic virus antiserum. The actual weights of precipitate obtained are shown in Table V, and the calculated weights for 1 ml. of antiserum are plotted against weight of antigen in Fig. 3. It will be seen that the curves for the two viruses are quite different, for aucuba mosaic virus with its own antiserum produces much more precipitate over the whole range tested than does tobacco mosaic virus. Difference also occurred in the ratios of antigen and antibody at the equivalence points. With tobacco mosaic virus equivalence was with 1 mg. of virus and 1 ml. of antiserum, whereas with aucuba mosaic virus equivalence was with 4 mg. of virus and 1 ml. of antiserum. The amount of antibody bound at equivalence with tobacco mosaic was only 0.4 mg. from 1 ml. of antiserum, whereas with aucuba mosaic virus 1.4 mg. of antibody were bound from 1 ml. of antiserum. After

removal of the precipitate formed by tobacco mosaic virus at the equivalence point, the supernatant fluid still reacted strongly with aucuba mosaic virus. Thus it seems that of the 1.4 mg. of antibody present in 1 ml. of the aucuba mosaic virus antiserum only 0.4 mg. is capable of reacting with antigens common to both aucuba mosaic and tobacco mosaic viruses, and 1 mg. is antibody specific to antigens in aucuba mosaic virus.

Variations in Rabbit Antisera.

Antisera obtained by immunizing different rabbits with the same antigen may vary greatly in their titres and in the amount of precipitate they form with a given amount of antigen. Two such sera (Nos. 11, 19) were obtained by injecting different rabbits with the same preparation of tobacco mosaic virus. When titrated against constant antigen, 0.001 per cent. tobacco mosaic virus solution, No. 11 gave a titre of 1/3000 and No. 19 one of 1/30. Experiments were therefore made to test whether this difference is only quantitative or whether it is in part qualitative, i.e. whether the two sera contained the same antibodies in different quantities or whether they also contained different antibodies. If they differed only quantitatively, it is apparent that serum No. 11 diluted 1/100 should be equal to No. 19 undiluted. When different amounts of tobacco mosaic virus were added to 1 ml. of No. 19 and to 1 ml. of No. 11 diluted 1/100 with 0.9 per cent. saline, however, quite different results were obtained (Table VI).

TABLE VI.—*Amounts of Precipitate Formed by Varying Amounts of Tobacco Mosaic Virus with Constant Amounts of Serum No. 19 and Serum No. 11.*

Weight of tobacco mosaic virus used (mg.).	The weight of precipitate (mg.) formed with—			Weight of precipitate (mg.) expected with 0.01 ml. of serum No. 11 according to curve A, Fig. 2.
	1 ml. of serum No. 19.	1 ml. of 1/100 serum No. 11 in saline.	1 ml. of 1/100 serum No. 11 in normal rabbit serum	
0.12	0.15	0.1	0.1	0.11
0.25	0.26	0.2	0.15	0.19
0.5	0.54	0.3	0.2	0.28
1.0	0.6	0.35	0.1	0.32
1.5	..	0.1	0	0.2
2.0	0.6	0	..	0
3.0	0.7
4.0	0.85

The total volumes adjusted to 5 ml.

The amount of precipitate with serum No. 19 increased much more rapidly with increasing amounts of antigen than with the diluted serum No. 11, and still produced precipitate without any tendency to decrease when the latter failed to give a precipitate because of antigen excess. This difference was not due to the greater concentration of protein in the undiluted serum, for when

serum No. 11 was diluted 1/100 with normal rabbit serum instead of saline, an even smaller precipitate resulted and antigen excess inhibition was apparent at a lower antigen concentration (Table VI). Thus it is obvious that the ratio of the weight of precipitate to the weight of antigen is different with the two sera, and that the difference cannot be explained merely on the basis of different quantities of the same antibodies.

AGGLUTINATION OF PEA NODULE BACTERIA (317) WITH ITS ANTISERUM.

Pea nodule bacteria 317 are Gram-negative rods, about 0.4μ wide and averaging 1.5μ long (variation 0.7μ to 3.5μ), and without flagella. 1 mg. of dried, well-washed bacteria, after killing with formalin, was found to correspond to 6×10^9 organisms counted in a haemocytometer. The weight of one bacterium, therefore, is approximately 1.7×10^{-13} g. The nitrogen content is about 5 per cent. of the dry weight.

In estimating the amount of antibody bound by the bacteria the globulin fraction and not the whole antiserum was used. Preliminary experiments showed that when 1 ml. of bacterial suspension containing 9×10^8 bacteria was mixed with 1 ml. of the globulin fraction solution diluted 1/400, no detectable agglutinin remained in the supernatant, whereas at a dilution of 1/200 the supernatant still contained agglutinin. It was assumed that these bacteria, like the viruses and the pneumococci studied by Heidelberger and Kabat (1937), would give a weight of precipitate proportional to the weights of antigen and antibody mixed over a range of dilutions, provided these two were kept in constant proportions. Therefore, in estimating the amount of antibody bound at equivalence, dense suspensions of bacteria were added to the globulin in the ratio of 9×10^8 bacteria to 1/400 ml. of the globulin fraction. These were then centrifuged and the protein bound by the bacteria estimated by measuring the decrease in colour given by the supernatant fluid with Folin's reagent. A control in which bacteria were mixed with globulin from normal rabbit serum was also included.

The details of the experiment were as follows: 2 ml. of the antiserum globulin diluted 1/10 (containing 1.76 mg. of protein) were mixed with (1) 3 ml. of bacterial suspension containing 7×10^{10} bacteria (12 mg. dry weight) and a second 2 ml. with (2) 3 ml. of saline. 2 ml. of normal serum globulin (also containing 1.76 mg. protein) were mixed with (3) 3 ml. of the bacterial suspension and a further 2 ml. with (4) 3 ml. of saline. The mixtures were kept for 2 hours at 37°C . and a further 2 hours at 1°C ., when the bacteria were centrifuged down from (1) and (3). 4.5 ml. of each mixture were then diluted to 20 ml. with water, and 3 ml. of 1.28*N* NaOH and 3 ml. of Folin's reagent diluted 1/3 were added to each. The solutions were allowed to stand for 5 minutes for the colour to develop, and then they were compared in a colorimeter. There was no difference between (3) and (4), showing that the bacteria had adsorbed no protein from the normal serum globulin. The colour ratio (1)/(2) was 43/46, showing that approximately 1/15th of the total protein had been bound by the bacteria. The supernatant fluid of (1) gave no reaction with further bacteria, so that all the agglutinin was bound. Thus, if it be

assumed that all the protein precipitated from the antiserum globulin was antibody, the amount of antibody precipitated at the equivalence point by 12 mg. of bacteria was 0.12 mg. and the antibody/antigen weight ratio was 0.01.

The result with the bacteria is obviously only approximate, for it is based on the assumption that all globulins, including the antibody, give the same intensity of colour with Folin's reagent. However, the more accurate micro-Kjeldahl method used for the viruses and by Heidelberger and Kabat (1934; 1937) for pneumococcus agglutination could not be used because of the small amount of antibody bound by the pea nodule bacteria. The amount of antibody bound by a given amount of bacteria can be increased if the amount of antibody is increased above that necessary for equivalence, but then not all the antibody is bound.

TABLE VII.—*Comparison of Antibody/Antigen Ratios at Equivalence with Molecular or Particular Weights of Antigen.*

Antigen.	Antibody/antigen ratio at equivalence.	Molecular weight of antigen or weight of antigen particle in units of molecular weight.
Ovalbumin	10.0 (1)	40,000 (5)
Horse-serum globulin	4.0 (2)	160,000 (5)
Fulgar (Busycon) haemocyanin	0.6 (3)	6,000,000 (5)
Bushy stunt virus	0.25-0.4 (4)	8,000,000 (6)
Tobacco mosaic virus	0.2 (4)	50,000,000 (7)
Pea nodule bacteria	0.01 (4)	10 ¹¹ (8)

(1) Taylor, Adair and Adair (1934). (2) Kleczkowski (1940). (3) Hooker and Boyd (1936). (4) Found in this study. (5) Svedberg and Pedersen (1940). (6) McFarlane and Kekwick (1938). (7) Kausche and Ruska (1939). (8) The average weight of a single nodule bacterium found to be 1.7 $\times 10^{-13}$ gm., thus 10¹¹ times greater than the weight of one hydrogen atom.

DISCUSSION.

Table VII shows the combining ratios for various antigens and antibodies at equivalence points, together with the molecular weights of the antigens or their weights in terms of the weight of a hydrogen atom. It can be seen that the antibody/antigen ratio decreases with increasing weight of the antigen particles, and that there is a smooth transition from egg albumin through haemocyanin and the plant viruses to the pea nodule bacterium. Thus the distinction between precipitation and agglutination becomes merely an arbitrary one of convenience, for there is no clear dividing line between the two. If the one is used for small particles forming stable solutions and the other for large particles forming unstable suspensions, it is obvious that the plant viruses can be placed in either with equal justification. The view that precipitation and agglutination are essentially the same process is strongly supported by the fact that the same antibody can sometimes act as either a precipitin or as an agglutinin; for example, Heidelberger and Kabat (1934) showed that

the same antibody could act as a precipitin for pneumococcal carbohydrate and an agglutinin for pneumococcus, and Jones (1927, 1928) showed that a precipitin for a protein agglutinated collodion particles coated with it.

Boyd and Hooker (1934) suggest that antibody/antigen ratios at equivalence are a function of geometrical relationship between antigen and antibody particles. It is obvious from Table VII that the ratio decreases consistently with increase in the weight and size of antigen particle, and it is possible that the number of antibody particles bound by an antigen particle is a function of antigen surface area and of area which can be covered by one antibody particle. However, so many assumptions about size, shape and degree of hydration of molecules or particles have to be made in such calculations, that the problem cannot profitably be discussed here.

Fewer differences than might have been expected were found between the behaviour of the rod-shaped tobacco mosaic and aucuba mosaic viruses on one hand and spherical bushy stunt virus on the other hand. In the region of antibody excess investigated no characteristic differences were found. In antigen excess, however, with the same weight of antibody about four times the weight of precipitate can be obtained with tobacco mosaic virus as with bushy stunt virus. Maximal precipitate with tobacco mosaic virus was obtained in much greater antigen excess, about 30 times the amount of virus needed for equivalence compared with 1.5 times for bushy stunt virus. Inhibition of precipitation because of antigen excess is also reached with much less bushy stunt virus than with tobacco mosaic virus. The results obtained with aucuba mosaic virus were similar to those obtained with tobacco mosaic virus.

The number of antibody molecules needed to cause precipitation is much less than that with which the large antigens can combine; for example, precipitation of tobacco mosaic virus can apparently result from the union of one virus particle with two antibody molecules, whereas at equivalence one particle unites with 70 antibody molecules, and in the region of antibody excess with still more.

The serological relationship between tobacco mosaic virus and tomato aucuba mosaic virus had previously been studied by Chester (1936) and Bawden and Pirie (1937). Chester found that in addition to the antigens the two viruses had in common each had specific antigens, but Bawden and Pirie found that aucuba mosaic virus contained all the antigens there were in tobacco mosaic virus, but in addition contained specific ones. Tests of the supernatant fluids in this work confirmed Bawden and Pirie, for after tobacco mosaic virus antisera were exhausted with aucuba mosaic virus they failed to react with tobacco virus, whereas after aucuba mosaic antiserum was exhausted with tobacco mosaic virus it still reacted with aucuba mosaic virus. In addition, it was found that of the total amount of antibody in an aucuba mosaic virus antiserum only about a third could be precipitated by tobacco mosaic virus, whereas the same amount of antibody could be precipitated by both viruses from tobacco mosaic virus antiserum.

However, the results comparing weak and strong antisera to tobacco mosaic virus show that different sera prepared against the same antigen can differ qualitatively in their antibody content, so the results of absorption

experiments cannot necessarily be taken as proof that two antigens are identical or different.

Antisera produced by rabbits injected with human serum were found to differ in the presence or absence of partial antibodies to different fractions of antigenic serum (Kleczkowski, 1938); strong sera contained antibodies to globulin and albumin whereas weak sera contained antibodies only to globulin. Tobacco mosaic virus antisera may similarly differ in their content of some partial antibodies.

The amount of antibody bound by pea nodule bacteria is much lower than that found by Heidelberger and Kabat (1934, 1937) for pneumococcus. This difference may be partly explained by the fact that only the amount of antibody bound at equivalence was determined for the pea nodule bacteria, and not the maximum amount that could be bound. However, the pneumococcus has a highly antigenic capsule with specific polysaccharide, and so might be expected to bind more antibody than the unencapsulated pea nodule bacteria.

SUMMARY.

Quantitative investigations of serological reactions of tobacco mosaic, aucuba mosaic, and bushy stunt viruses and a strain of pea nodule bacteria, with their homologous antisera, and of cross-reaction between tobacco mosaic and aucuba mosaic viruses, were made.

Antibody/antigen ratios in the precipitate formed at equivalence point by these plant viruses with their homologous antisera occupy an intermediate position between ratios for bacterial agglutination and for precipitation of smaller antigens like ovalbumin or blood serum proteins.

An aucuba mosaic virus antiserum contained antibodies reacting with aucuba mosaic virus but not with tobacco mosaic virus, in addition to antibodies reacting with both, whereas all antibodies in a tobacco mosaic virus antiserum reacted with both viruses.

With the same amount of antibody maximum precipitate with the rod-shaped tobacco mosaic and aucuba mosaic viruses is much greater than with the spherical (or almost spherical) bushy stunt virus, and is formed in much greater antigen excess.

Qualitative differences between strong and weak tobacco mosaic virus antisera were found.

I wish to express my gratitude to Mr. F. C. Bawden for supplying the virus solutions and the aucuba virus antiserum.

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THE SEROLOGICAL REACTIONS OF VIRUSES CAUSING TOBACCO NECROSIS.

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THE purification of tobacco necrosis virus has been described by Pirie, Smith, Spooner and MacClement (1938). Their method involved precipitating the virus from infective sap by the addition of three volumes of alcohol followed by fractional precipitation with ammonium sulphate, and their end-product always consisted of two fractions. One was a crystalline nucleoprotein, which sedimented in the ultra-centrifuge with a single sharp boundary corresponding to $S_{w20^\circ} = 130 \times 10^{-13}$ cm. sec.⁻¹ dynes⁻¹. The other fraction was chemically similar but was amorphous, and in the ultra-centrifuge was inhomogeneous, the principal components giving boundaries corresponding to $S_{w20^\circ} = 58 \times 10^{-13}$ and 220×10^{-13} . No explanation was put forward to explain these unusual results, but there seemed two that were most likely. One was that the heterogeneous end-product was a result of the method of purification, and the second that the virus culture was a mixture containing viruses with particles of different sizes, not all of which would crystallize. The work described in this paper was started to test these possibilities.

MATERIALS AND METHODS.

In most of the work two cultures of virus were used. One was originally supplied by Dr. Kenneth M. Smith in 1936, and had been maintained for four years at Rothamsted. The second was supplied by Dr. Smith from Cambridge in 1940, and was directly descended from that used by Pirie *et al.* (1938). These will be referred to as the Rothamsted and Cambridge cultures respectively. In addition, a few tests were made with virus found in naturally infected tobacco plants.

Unfortunately no plants are known whose leaves are infected systemically by tobacco necrosis virus, and the restriction of multiplication to local lesions

greatly hampers the production of virus in large quantities. The plants used were tobacco, var. White Burley, and French bean (*Phaseolus vulgaris*), var. Canadian Wonder. The same inoculum produces more lesions per unit area on bean than on tobacco leaves, but, in spite of this apparently greater susceptibility, the virus content of expressed sap from tobacco leaves is considerably higher than that from beans. Tobacco plants were therefore used for the production of virus and beans for the infectivity tests. When two preparations were being compared they were rubbed over opposite halves of the same



FIG. 1.—Leaf of French bean (*Phaseolus vulgaris*) infected with two serologically unrelated tobacco necrosis viruses. The left-hand half of the leaf was rubbed with the Rothamsted culture and the right-hand half with the Cambridge culture.

leaves, and when there were more than two each was rubbed over at least six half leaves chosen at random. Both bean and tobacco become increasingly resistant with age, and few lesions are obtained on old plants.

All the virus cultures used produced apparently identical symptoms (Fig. 1), both on tobacco and bean, but when rubbed to opposite halves of the same leaves the Rothamsted culture produced rather more lesions than the Cambridge one on beans and less on tobacco.

Three antisera were used. One, that used by Pirie *et al.*, was supplied by Mr. E. T. C. Spooner. The others were prepared by Dr. A. Kleczkowski, one

against the Rothamsted and the other against the Cambridge culture. The rabbits were injected twice at five-day intervals with 1 mg. of purified virus, and were bled ten days after the second injection. All the antisera gave precipitin titres in excess of 1 in 500. The precipitin tests were made in tubes of 7 mm. diameter, which were kept in an illuminated water bath at 50° C. Usually 1 c.c. of serum at a constant dilution was added to a series of tubes, each containing 1 c.c. of antigen solution of different concentration, but some tests were made with constant antigen and varying serum.

The virus was purified in two ways. One was that described by Pirie *et al.* This readily gives a colourless product of high serological activity, but it involves the handling of large volumes of alcohol and the infectivity of the final product was found to vary widely. The following method gives an end-product whose chemical, physical and serological properties are apparently identical with those of the virus prepared by the other method, but its infectivity is more constant, and weight for weight is approximately as infective as virus in frozen, clarified sap.

Sap is expressed from necrotic tobacco leaves, frozen, thawed and centrifuged. Ammonium sulphate is added to the clear supernatant fluid at the rate of 250 g. per litre, and the resulting precipitate centrifuged off. This is resuspended in a volume of water equal to one-tenth of the original sap, brought to pH4 by the addition of acetic acid and centrifuged. The supernatant fluid is one-quarter saturated with ammonium sulphate, allowed to stand for an hour and then centrifuged. The precipitate is suspended in a volume of water equal to one-fiftieth of the original sap, and centrifuged to get rid of insoluble material. Saturated ammonium sulphate solution is now added to the supernatant fluid slowly until it becomes opalescent at room temperature, when it is placed at 0° C. After a few hours the preparation is centrifuged while cold, the precipitate is discarded and the supernatant fluid allowed to warm up to room temperature. The precipitate that now separates contains all the virus: it is sedimented, resuspended in water, adjusted to pH6 with dilute NaOH, and any insoluble material is centrifuged off. The fractional precipitation with ammonium sulphate at 0° C. and room temperature is then repeated at pH6. The precipitate separating at room temperature is taken up in a small volume of water and dialysed. This sometimes produces a small precipitate, which is centrifuged off. The final preparation is colourless and slightly opalescent. 10⁻⁹ g. are usually sufficient to give lesions in beans, and 1 c.c. containing from $\frac{1}{3} \times 10^5$ to $\frac{1}{6} \times 10^5$ g. gives a precipitate visible to the naked eye when mixed with 1 c.c. of antiserum at 1/50.

SEROLOGICALLY UNRELATED VIRUSES AS THE CAUSE OF TOBACCO NECROSIS.

The first experiments were made with the Rothamsted virus culture. This was purified by the two methods to see whether the method of purification affected the homogeneity of the end-product. If the end-product separated into crystalline and amorphous fractions it was also intended to inoculate these separately to plants to test the possibility of their being separate strains, i. e. it was intended to see whether crystallization could be used as a method

of separating strains, and whether a wholly crystalline inoculum would give rise to a wholly crystalline virus preparation.

Both methods of purification gave the same end-product, one which would not crystallize, although many attempts to make it do so were made under the conditions described by Pirie *et al.* (1938). Samples were sent to Mr. N. W. Pirie, who also failed to obtain any crystals, and who found the preparation to behave like the amorphous fraction of his earlier preparations. This result seemed a fairly definite indication that there were different viruses or strains causing tobacco necrosis, some of which would crystallize and others not, and that the Rothamsted culture contained only the latter. The experiment was therefore repeated with the Cambridge virus culture. Again, however, the same result was obtained; regardless of the method of purification only an amorphous end-product was obtained. Both cultures have since been purified many times and no crystalline material has ever been isolated.

At first this seemed to make the proof of the existence of virus strains impossible, but when purified preparations of the Cambridge and Rothamsted cultures were tested against the three antisera differences were immediately detected between them, and positive evidence was obtained that the culture used by Pirie *et al.* was a mixture. The result of one such test is given in Table I. Preparations of both the Rothamsted and Cambridge cultures reacted with the serum used by Pirie *et al.* in 1938, but the Rothamsted culture failed to react with the serum made against the Cambridge culture in 1940

TABLE I.—*Precipitin Tests with Purified Preparations of the Rothamsted and Cambridge Virus Cultures and Three Antisera.*

A. *Rothamsted culture of tobacco necrosis virus as antigen.*

Antiserum.	Time.	Dilution of antigen.						
		1/1.	1/2.	1/4.	1/8.	1/16.	1/32.	1/64.
Prepared in 1938 by Pirie <i>et al.</i>	10 mins.	+	++	+	—	—	—	—
	30 „	+++	+++	++	+	—	—	—
	180 „	++++	++++	++++	++++	+++	++	+
Against the Rotham- sted culture	30 mins.	—	—	—	+	—	—	—
	180 „	—	++	++++	++++	+++	++	+
Against the Cambridge culture	24 hours	—	—	—	—	—	—	—

B. *Cambridge culture of tobacco necrosis virus as antigen.*

Prepared in 1938 by Pirie <i>et al.</i>	10 mins.	+	+	—	—	—	—	—
	180 „	++++	++++	++++	++++	+++	++	+
Against the Rotham- sted culture	24 hours	—	—	—	—	—	—	—
Against the Cambridge culture	10 mins.	—	—	+	—	—	—	—
	180 „	++	++++	++++	++++	+++	++	+

+ signs indicate the degree of precipitation; — indicates that there was no precipitate. The dilution of antiserum was constant at 1/50 in 0.85 per cent. NaCl solution in all tests. 1/1 in the dilution of antigen equals 0.2 mg. of purified virus per c.c. Note that the position of the precipitation optima show that the antisera prepared against the Cambridge and Rothamsted virus cultures were richer in antibodies specific to them than the serum prepared against the mixed culture.

and the Cambridge culture failed to precipitate with the antiserum to the Rothamsted culture. Thus it was obvious that the disease tobacco necrosis can be caused by serologically unrelated viruses, and that the culture used by Pirie *et al.* contained at least two distinct viruses now separated into the Rothamsted and Cambridge cultures. As both of these failed to crystallize, it seems likely that the crystalline material represents yet another strain. As the two cultures used in this experiment originally came from the same source in 1936, the simplest explanation that can be offered for these results is that the culture then contained at least three viruses, one crystalline and the others amorphous, and that in continued sub-culturing the crystalline virus has been eliminated from both cultures, while one of the amorphous viruses has been eliminated at Rothamsted and the other at Cambridge.

Unfortunately it has not been possible to make any determinations of the sizes of the virus particles present in the two virus cultures, but the fact that the material purified by Pirie *et al.* gave a number of different sedimentation constants suggests that the serologically distinct viruses also have different particle sizes. Smith and MacClement (1940) found that tobacco necrosis virus behaved differently from any other studied in its filterability through collodion membranes. Although its filtration end-point was constant around 40 m μ ., there was always a sharp drop in the infectivity of filtrates through membranes with pores of diameter between 125 m μ . and 250 m μ .. This result is more simply explained by the theory that their virus culture was a mixed one, containing particles of various sizes, than on the theory of a reversible aggregation of particles of a uniform size, which they suggest. A culture of mixed viruses, in which the proportions of viruses with particles of different sizes was continually fluctuating, would also satisfactorily explain why the pore sizes of membranes causing this drop in infectivity varied with different batches of virus. Indeed it is probable that the change in filterability over a period of some months, noted by Smith and MacClement (1940), is an example of the unconscious selection of certain viruses from a mixture during continued culturing, such as that postulated above to explain the production of the Rothamsted and Cambridge cultures from a common stock.

Serological tests with cultures of virus causing tobacco necrosis found in naturally infected tobacco plants suggest that most of such cultures are mixtures and that the Rothamsted and Cambridge ones are also mixtures. Of five such infections tested, the virus from one reacted only with antiserum against the Rothamsted culture, but the other four reacted with serum against both cultures. The results of a test with one of these four are shown in Table II. This culture contains considerably more antigen capable of reacting with the antiserum to the Rothamsted than to the Cambridge culture. This is shown by the higher precipitation titre given in the constant antiserum test with the former serum, and by the large zone of inhibition by antibody excess in the constant antigen test with the antiserum to the Cambridge culture, which does not occur with the antiserum to the Rothamsted culture. In precipitation tests with mixtures of serologically related strains of other viruses such differences have never been noticed, for, although widely different precipitation optima may be obtained with different sera, the precipitation end-point in constant antisera tests is usually the same with homologous and heterologous

TABLE II.—*The Serological Reactions of a Virus Culture found in a Naturally Infected Tobacco Plant.*A. *Constant antiserum test (serum dilution 1/40).*

Serum.	Time.	Antigen dilution.						
		1/1.	1/2.	1/4.	1/8.	1/16.	1/32.	1/64.
Against the Rothamsted culture	1 hour	.	+++	++++	+++	++	+	—
	4 hours	.	+++	++++	++++	+++	++	+
Against the Cambridge culture	1 hour	.	+	—	—	—	—	—
	24 hours	.	+++	++	+	—	—	—

B. *Constant antigen test (antigen dilution 0.05 mg. per c.c.).*

Serum.	Time.	Antiserum dilution.				
		1/10.	1/20.	1/40.	1/80.	1/160.
Against the Rothamsted culture	0.5 hours	.	+++	++	+	—
	24 „	.	++++	++++	++++	+++
Against the Cambridge culture	0.5 hours	.	—	—	—	—
	24 „	.	—	—	—	+

The sample of virus used was purified, and in the constant antigen test antigen dilution 1/1 equals 0.2 mg. of purified virus per c.c.

antisera. With other viruses precipitation titres have been found to be extremely valuable for quantitative work, but it is apparent from Table II that unless it is known that the serum contains antibodies to all the viruses present in the culture, the precipitation end-point of tobacco necrosis virus preparations may be little indication of the total amount of virus present.

Further evidence for the complexity of the virus cultures in naturally infected plants is the frequent occurrence of double zones in the precipitation tests. The proportions in which serologically distinct viruses occur in an infection may vary widely. Table III shows the effect of concentrating the virus from one naturally occurring infection. The clarified sap gave only one zone of precipitation and a titre of 1/8. After precipitating the virus

TABLE III.—*The Effect of Concentrating the Virus from a Natural Infection and the Production of Double Zones of Precipitation.*

Antigen.	Time.	Dilution of antigen.				
		1/1.	1/2.	1/4.	1/8.	1/16.
Clarified sap	30 mins.	—	+	—	—	—
	60 „	—	++	+	—	—
	120 „	+	+++	++	+	—

Antigen.	Time.	Dilution of antigen.						
		1/25.	1/5.	1/10.	1/20.	1/40.	1/80.	1/160.
Virus concentrated by (NH ₄) ₂ SO ₄	30 mins.	+	—	—	—	+	—	—
	60 „	++	+	—	+	++	+	—
	120 „	+++	+++	+	++	++++	++	+

The antiserum was that prepared against the Rothamsted virus culture and was used at a dilution of 1/50.

with ammonium sulphate and redissolving in a volume of water equal to one-twentieth of the sap, the preparation gave two zones of precipitation and a titre of 1/160. Here it is apparent that of the two strains present in the culture only one was present in the sap in sufficient quantity to give a reaction, whereas the other still reacted when the sap was diluted 1/8. Such double zones have never been seen in precipitation tests with other viruses which exist in strains, although some of these have been shown to be complex antigens, and their occurrence strongly suggests the presence of serologically distinct entities. It is possible that tobacco necrosis virus particles are themselves complex antigens, and this could explain some of the results with the naturally occurring infections. For example, if the Rothamsted culture contained only one type of virus particle these may have two antigenic groupings, A and B, and similarly the Cambridge culture might contain only one particle, but with two antigenic groupings, C and D. Then it is obvious that another culture containing a single virus with antigenic groupings B and C would react with antisera to both the Cambridge and Rothamsted cultures. However, as a virus particle must presumably precipitate as a whole and not as individual antigenic groupings, it is difficult on this theory to account for the widely different precipitin titres with different antisera (Table II). This possibility could only be tested by isolating numerous cultures of tobacco necrosis virus from single local lesions, in the hope that such cultures would contain only one type of virus particle, but even then the possibility of mutations that would alter the serological reactions would always arise. At present the results are most satisfactorily explained by regarding the viruses as simple and unrelated antigens.

THE INACTIVATION OF TOBACCO NECROSIS VIRUSES.

The type of precipitate given by all cultures of tobacco necrosis viruses has been the same and resembles that formed by tomato bushy stunt virus. The floccules are small and dense, and settle to form compact masses. Tobacco mosaic virus, on the other hand, forms loose nebulous floccules resembling those formed by bacterial flagella. Bawden and Pirie (1938) suggested that the difference between the precipitates of tobacco mosaic and bushy stunt viruses might result from the fact that the former has rod-shaped particles and the latter spherical ones. Work with further viruses has supported this suggestion, for of ten studied serologically, seven have been found to show anisotropy of flow, and so have anisodimensional particles, and all of these form the flagellar type of precipitate. Three do not show anisotropy of flow, those causing tomato bushy stunt, tobacco necrosis and tobacco ringspot, and so presumably have spherical or quasi-spherical particles, and these all give the same type of compact precipitate.

Tobacco necrosis also resembles bushy stunt virus (Bawden and Pirie, 1940) in that it is readily rendered non-infective without losing its serological activity. With viruses such as tobacco mosaic and potato "X" the separation of these two specific activities has been produced at all easily only by treatment with formalin, nitrous acid and hydrogen peroxide or by irradiation with X-rays or ultra-violet. With tobacco necrosis and bushy stunt viruses

the separation is produced by ageing, alkali and heating. Sap from plants suffering from tobacco necrosis loses its infectivity fairly rapidly at room temperature and is usually non-infective after 2-3 months, the exact time depending on the original virus content and the temperature. From such samples of old sap a non-infective protein can be isolated, which has physical and serological properties that are apparently identical with those of fully active virus preparations. Sap from healthy plants, of course, does not contain such a protein either before or after ageing. It is unknown what changes occur within the particles to cause them to lose their infectivity, and no tests have been made to determine whether bacteriologically sterile filtrates of infective sap or solutions of purified virus would also lose their infectivity on standing at room temperature.

Purified preparations of both cultures of tobacco necrosis viruses lose their infectivity without otherwise altering their properties when treated with dilute alkali. The results of one experiment with the Rothamsted culture are given in Table IV, from which it will be seen that most of the infectivity was destroyed after 24 hours at 18° C. and pH 8, and all of it at pH 9, whereas at pH 10 there was no change in either the precipitation end-point or the optimal precipitation point. The non-infective preparations are fully active antigenically, and do not merely behave as haptens, for a rabbit injected with an alkali-inactivated preparation produced an antiserum that reacted in exactly the same manner as one prepared against a fully active preparation of the same virus.

TABLE IV.—*The Dissociation of Infectivity and Serological Activity by Alkali.*

pH.	Average number of lesions per half leaf.	Serological titre.
6	270	1/400,000
8	4.2	1/400,000
9	0	1/400,000
10	0	1/400,000

The virus samples used were from a purified preparation of the Rothamsted culture. Virus at 0.2 mg. per c.c. was exposed to the pH stated for 24 hours, then neutralized and tested. Infectivity tests were made at a virus content of 0.05 mg. per c.c., and the serological titre is the smallest weight of virus giving a visible precipitate.

The rate at which infective sap loses infectivity is greatly increased by heating, and sap that takes some weeks to become inactive at room temperature is inactivated by a few minutes at 70° C., some hours at 50° C., and days at 38° C. The effect of heating has been studied in greater detail with purified preparations, and it has been found that the loss of infectivity produced by heating at temperatures below about 85° C. has no effect on the serological reactions. Table V shows the results of heating crude infective sap and a purified preparation of the Rothamsted culture of tobacco necrosis virus at pH 6 for ten minutes at various temperatures; the effects of heating a purified preparation of the Cambridge virus culture in pH 6 phosphate buffer for various times at 50° C. are also given. Only at 90° C. was there any fall in the serological titre, and at this temperature denatured protein separated from the

TABLE V.—*Effect of Heat on Tobacco Necrosis Viruses.*A. *Heating the Rothamsted culture for ten minutes at various temperatures before and after purification.*

Temperature.	Crude sap.		Purified preparation.	
	Average number of lesions per half leaf.		Average number of lesions per half leaf.	Serological titre.
Control	280		300	1/400,000
50° C.	109		216	1/400,000
60° C.	3		33	1/400,000
70° C.	1		1.5	1/400,000
80° C.	0		0	1/400,000
90° C.	0		0	1/400,000

B. *Heating a purified preparation of the Cambridge culture for various times at 50° C.*

Time of heating.	Average number of lesions per half leaf.	Serological titre.
Control	205	1/500,000
0.25 hours	111	1/500,000
1 hour	45	1/500,000
4 hours	20	1/500,000
16 "	1	1/500,000

All samples were heated in 0.1m. phosphate buffer at pH 6.

TABLE VI.—*Correlation between Loss of Infectivity and Serological Activity on Heating Potato Virus "X" for Ten Minutes at Various Temperatures.*

Temperature.	Serological titre.	Average number of lesions per half leaf at—	
		1/2.	1/20.
Control	1/256	230	108
59° C.	1/256	215	94
62° C.	1/128	130	29
65° C.	1/8	16	2.5
68° C.	No precipitate	0	0

The samples were heated in 0.1 m. phosphate buffer at pH 6. The infectivity tests were made on tobacco plants, the samples being tested at two dilutions. The serological titre is the greatest dilution at which 1 c.c. produced a visible precipitate when mixed with 1 c.c. of antiserum at 1/50.

preparation. The thermal inactivation point for ten minutes' heating was the same for both virus cultures. It was also the same for virus in crude sap and for purified preparations, but the infectivity of the former was reduced more by heating in the neighbourhood of 60° C., probably because of the separation of a precipitate of denatured plant proteins. It lies between 70° C. and 80° C., which agrees with the value given by Smith and Bald (1935), though it is considerably lower than that found by Price (1938).

The behaviour of tobacco necrosis on heating contrasts remarkably with that of potato virus "X." Table VI shows the results of heating this virus in

pH 6 phosphate buffer for ten minutes at various temperatures, and it will be seen that the loss of infectivity and of serological activity are parallel. Indications that a slight separation of infectivity from serological activity can occur with virus "X" were obtained by heating at pH 6 for long periods at 50° C., for after 16 hours the infectivity was reduced to one-quarter, whereas the precipitation end-point was only reduced to three-quarters of the control. This effect, however, is very small compared with tobacco necrosis virus (Table V), whose infectivity was reduced to 10 per cent. in four hours and to less than 0.05 per cent. of the control in 16 hours at 60° C., whereas the serological activity was unaffected. Differences in the behaviour of the two viruses are also clearly shown by the range of temperatures over which heating for ten minutes produces detectable effects. Tobacco necrosis, which is not completely inactive after ten minutes at 70° C., has its infectivity considerably reduced after ten minutes at 50° C. By contrast, potato virus "X" is not significantly affected by ten minutes at 59° C., but is completely inactivated by ten minutes at 68° C. It is apparent that the processes leading to loss of infectivity in the two viruses are different, for the inactivation of tobacco necrosis virus has a small temperature coefficient, and that of potato virus "X" a large one. Large temperature coefficients are characteristic of protein denaturation, and that this is closely linked with the inactivation of virus "X" is shown by the separation of a precipitate from the heated samples as they lose infectivity, and by the loss of anisotropy of flow. With all the plant viruses that have been studied, loss of serological properties has only been produced by treatments causing protein denaturation, whereas loss of infectivity can often occur as a result of slight intramolecular changes that have little effect on the gross physical and chemical properties. It seems that some such intramolecular changes occur in particles of tobacco necrosis when heated, treated with alkali, or aged in sap, whereas with potato virus "X" these treatments tend to cause the disruption of the particles.

DISCUSSION.

Serological methods have been of greatest value in plant virus studies in showing that viruses causing different symptoms may contain common antigens and so are related strains. The work described here shows that they can also be valuable in demonstrating that a disease, previously regarded as caused by a single entity, may be caused by a number of viruses. It has been known for some years that viruses with no common antigens can cause identical clinical symptoms in animals, for example foot-and-mouth disease, and this has been a complicating factor in their control, but no such diseases were known in plants. The demonstration that tobacco necrosis can be caused by a number of serologically unrelated viruses, therefore, is a further indication of the similarity between the viruses now too often separated into the two groups, plant and animal viruses. From what is already known it is obvious that the viruses placed in each group have widely different properties, and it seems probable that some of those attacking animals may have more in common with some plant viruses than with other animal viruses, and *vice versa*. The results described in this paper show that of two plant viruses, tobacco necrosis

and potato "X," one readily becomes non-infective while retaining its serological activity, but the other does not. It is likely that similar differences also exist between animal viruses, for such differences would explain why the heating or ageing of viruses produces successful vaccines with some, but not with others.

The serologically unrelated viruses causing tobacco necrosis used in this work all have similar stabilities and general physical properties, but it has already been suggested that they may differ in particle size, and it is possible that the disease can also be caused by viruses with other properties. For example, all the preparations described here gave amorphous precipitates in conditions similar to those in which Pirie *et al.* (1938) obtained crystals, and the virus used by Price (1938 ; 1940) and Price and Wyckoff (1939) appears to differ from any studied in England. This seems to be a homogeneous culture, for preparations sedimented in the ultracentrifuge with a single boundary corresponding to $S_{w20} = 112 \times 10^{-13}$ cm. sec. $^{-1}$, dynes $^{-1}$, a value different from any found by Pirie *et al.* It also seems to be more resistant to heat and to alkali, for it was not completely inactivated after ten minutes at 90° C. or 24 hours at pH 9. It is noticeable, however, from Price's data that heating for ten minutes at temperatures considerably lower than the thermal inactivation point greatly reduced infectivity, and Price (1939) comments on the small temperature coefficient of thermal inactivation of tobacco necrosis virus compared with that of tobacco mosaic virus. The value of 4 for Q_{10} , which he obtained, is almost certainly too small for the inactivation to have been a result of protein denaturation, but as he made no serological tests it is impossible to be sure that his culture behaved like those described in this paper.

It is difficult to decide what, if any, relationship these various viruses causing tobacco necrosis have with one another. In the past it has often been found that symptoms are quite unsuitable as a basis for identifying and classifying viruses, and instead their properties *in vitro* have been increasingly used. Serological reactions in particular have proved useful for demonstrating relationships, and it has become generally recognized that viruses reacting with each other's antisera are related strains and those that do not are unrelated. As these serologically unrelated viruses causing tobacco necrosis can differ in other than antigenic properties, it would seem preferable, for the present at least, to continue this practice and to regard them as separate viruses rather than related strains, although they cause identical diseases.

SUMMARY.

A method is described for the purification of tobacco necrosis viruses that leads to little or no loss of infectivity. Evidence is presented showing that tobacco necrosis is a disease that can be caused by a number of serologically unrelated viruses. All the viruses used failed to crystallize, and it is shown that the virus culture from which Pirie *et al.* (1938) obtained crystals was a mixed one. It is suggested that the serologically distinct tobacco necrosis viruses have particles of different sizes, and that they may differ in stability. The behaviour of the tobacco necrosis viruses on heating, ageing or treating

with dilute alkali differs from that of potato virus "X," for the former are rendered non-infective without being denatured, and they remain fully active antigenically.

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SOME PROPERTIES OF TOBACCO ETCH VIRUSES

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(With Plate 6)

ALTHOUGH the tobacco etch viruses were discovered 13 years ago and have since been reported as of economic importance in various parts of the world, their properties have been little studied. Johnson (1930) differentiated four strains on the basis of the symptoms they caused, and gave a list of susceptible hosts, all members of the Solanaceae. He found that severe etch virus was inactivated when infected leaves were dried. Chester (1935) stated that severe etch virus was serologically related to tobacco mosaic virus, but later found that this result was due to a contamination (1937) and that the etch viruses formed a serological group distinct from any other viruses. Stanley & Wyckoff (1937) were able to sediment severe etch virus from clarified sap by high-speed centrifugation; the rate of sedimentation was about the same as that of tobacco mosaic virus, but the boundaries were too diffuse for accurate measurements. Lauffer & Stanley (1938), without giving any further details of methods of preparation or other properties, placed this virus among those showing the phenomenon of anisotropy of flow. Kassanis (1939) showed that severe etch virus was transmitted by *Myzus persicae* Sulz.: he also found that it differed from any plant virus previously studied in that it caused the formation of crystalline intranuclear inclusion bodies.

The study of the properties of the etch viruses described in this paper was undertaken primarily in the hope that it would give some indication of the mechanism of formation of the specific intracellular inclusions. However, no differences have been found between these and other viruses that can account for their different behaviour in vivo. Two strains of tobacco etch virus were used; these were kindly supplied by Drs W. M. Stanley and W. C. Price of the Rockefeller Institute, Princeton, under the names severe etch virus (S.E.V.) and mild etch virus (M.E.V.) respectively.

SYMPTOMATOLOGY

Quantitative infectivity tests with the etch viruses were made by local lesions, using a modification of the starch-iodine method devised by Holmes (1931) for tobacco mosaic virus. Five to seven days after inoculation the plants were placed in the dark from the afternoon to the next morning. The inoculated leaves were then picked, killed by being plunged into boiling water, decolorized with warm 95 % ethyl alcohol, washed in tap water and treated with a solution of iodine in potassium iodide. Under good conditions the lesions now show clearly as black areas against a white background, even in leaves which previously showed no signs of lesions (Pl. 6, fig. 1). No definite time for leaving plants in the dark can be given, for it varies with the weather conditions controlling the photosynthetic activities of the plants. The form of the iodine lesions varies. Most often they are thick black rings with white centres, but sometimes they are well-defined solid spots and occasionally the spots have diffuse edges. The presence of the virus impedes the translocation of starch both

from the actual entry points and from other tissues invaded. Thus, by decolorizing and staining the inoculated leaves at various intervals after infection the path taken by the virus from the entry point is clearly shown. It first spreads slowly from the entry point to the nearest minor vein, down which it moves more rapidly to the main vein and the petiole; there is little or no movement in any other direction.

As the starch-iodine method is laborious and does not give good results unless the times for each treatment are exactly right, a search was made for a host that might give visible countable local lesions. This failed. None of the plants examined outside the Solanaceae took the virus, and *Solanum nodiflorum* and *S. Melongena* were also immune. *Hyoscyamus niger*, *Datura Stramonium*, *Lycopersicum esculentum* and twelve species of *Nicotiana* were all susceptible, but reacted to S.E.V. in much the same way as tobacco, var. White Burley. Sometimes the rubbed leaves showed quite definite necrotic rings similar to the local lesions produced by the S strain of potato virus X (Salaman, 1938) (Pl. 6, fig. 2). More often they showed faint, diffuse chlorotic spots that could not be counted accurately (Pl. 6, fig. 3), and sometimes showed no obvious symptoms. It seemed that the type of local reaction depended on the weather conditions, but the exact conditions determining them were not found.

The systemic symptoms of infection with S.E.V. are more constant. They appear from 6 to 10 days after infection and first take the form of a clearing of the veins with a fine necrotic etching. The symptoms first appear at the bases of the leaves and spread upwards (Pl. 6, fig. 4). The young, actively growing leaves usually soon have symptoms all over, but some of the older leaves frequently have pronounced symptoms at the bases while the tops look quite normal. In such leaves the external symptoms are correlated with the distribution of the virus, for no infection is obtained when the healthy looking parts of such leaves are used as inoculum.

The vein clearing and etching persist for a few days and are then replaced by a general pronounced chlorosis (Pl. 6, fig. 5). The subsequent growth of plants is much reduced. Although the same number of leaves are produced as in healthy plants, they are much smaller, have a bright mottle and tend to lie horizontally while their margins turn downwards and inwards. The internodes are considerably shorter than usual, so that the whole plant has a stunted appearance.

In the work on insect transmission the infected plants frequently produced greatly distorted leaves. Sometimes the lamina developed on one side only of the main vein and sometimes there was no appreciable lamina at all, when the leaf consisted of little but the main vein and resembled a tendril. At first it was thought that this symptom was caused by a distinct strain of the virus, which had been separated from a mixture by the aphides, but attempts to reproduce the symptoms by inoculation failed until small seedlings similar to those used in the insect work were infected. Then it was found that the deformation is a constant feature of S.E.V. infections in young seedlings.

The symptoms of mild etch take about 2 days longer to appear than those of severe etch. Inoculated leaves usually show diffuse chlorotic areas, and the first systemic symptoms are a milder vein clearing and etching. When these symptoms fade they are replaced by a mild interveinal mottle with green vein-banding, much less definite than the chlorosis of severe etch. Bawden (1939) suggested that the great stunting of plants infected with S.E.V. might be a result of the presence of nuclear abnormalities in the form of crystalline plates. However,

this now seems unlikely, for plants infected with M.E.V. are almost as large and well developed as healthy plants and yet their nuclei also contain plate-like inclusions.

Both types of intracellular inclusion, the cytoplasmic and intranuclear, described with S.E.V. (Kassanis, 1939) have been found constantly in plants infected with M.E.V., but they show some characteristic differences. Although, as with infections with S.E.V., all infected tissues contain inclusions, they are fewer. With S.E.V. the nuclei are often so packed with inclusions that they cannot be counted, whereas with M.E.V. the nuclei usually contain only one or two inclusions and the largest number ever found was eight (Pl. 6, fig. 6). The average size of the inclusions produced by M.E.V. is greater, the sides averaging about 8μ long instead of 5μ . Kassanis (1939) stated that the plates showed no extinctions in the polarizing microscope, and it was suggested that this might be because of their small size. When examined between crossed Nicol prisms the intranuclear inclusions formed by M.E.V. are clearly birefringent with straight extinction when viewed edgewise, but not when viewed flat. A more detailed examination of the intranuclear inclusions in plants infected with S.E.V. has now shown that these are similarly birefringent. Not all the intranuclear inclusions formed by M.E.V. seem to be flat plates, for many show a characteristic cross suggesting that they may be eight-sided bi-pyramids (Pl. 6, fig. 7). The larger size and more definite shape of the M.E.V. inclusions is probably a direct result of their smaller number. With S.E.V. it seems that many crystalline foci arise in each nucleus and the resulting crystals compete both for material and space, whereas with M.E.V. there are only a few foci from which crystal growth continues more slowly and free from competition.

Cytoplasmic inclusions of the *X*-body type are common in infections with M.E.V., although they may not occur in every cell as do the intranuclear inclusions. They are definitely granular, like those produced by S.E.V., but are smaller. The cytoplasmic inclusions are usually amorphous, but in the characteristically malformed leaves produced by infecting young seedlings with S.E.V. these inclusions tend to crystallize. This crystalline phase seems to be a secondary stage in the history of the *X*-bodies, and shows itself by the production within the granular bodies of needle-shaped, birefringent bodies varying in length from 2 to 10μ (Pl. 6, fig. 8). Whether these are true crystals or paracrystalline fibres, like those produced by precipitating tobacco mosaic virus with acid, is unknown. These birefringent needles have been seen in epidermal strips from malformed leaves within one month of infecting young seedlings, but they usually require longer than this and seem to develop as a result of ageing of the inclusion. All the *X*-bodies in a malformed leaf do not necessarily contain crystals of this type, but the greater the malformation the greater is the tendency to crystallize. Some parts of large *X*-bodies may contain these needles while other parts remain amorphous and granular. When epidermal strips are mounted in weak acid the needles disappear and the *X*-bodies regain their granular appearance. In addition to tobacco, such crystallized *X*-bodies have been found in plants of *Nicotiana glutinosa* and *Datura Stramonium*, which were infected with S.E.V. when seedlings and developed malformed leaves. *X*-bodies containing similar, though smaller, needles have also been found in leaves of tobacco plants that had been infected with M.E.V. for a long time, although they were not malformed, and in malformed leaves of *Nicotiana glutinosa* infected with M.E.V. A somewhat similar tendency of old *X*-bodies to crystallize has been reported by Henderson Smith (1930) and Sheffield (1934) with aucuba mosaic virus, but here the crystals are large plates and not small needles.

IMMUNOLOGICAL REACTIONS AND INTERACTIONS WITH OTHER VIRUSES

To facilitate quantitative work and to study the possible relationships between S.E.V. and other plant viruses antisera were prepared against it. Two methods of immunizing rabbits were used. One rabbit received four intravenous injections at 5 day intervals of 2 c.c. of a concentrated virus preparation made by precipitation with ammonium sulphate, and a second rabbit received five intravenous injections of 2 c.c. of infective sap clarified by a few minutes centrifugation at 16,000 rev./min. The second rabbit showed signs of anaphylactic shock, but both produced antisera that precipitated at dilutions greater than 1/200.

Using the antisera at a dilution of 1/50 in 0.85% NaCl solution good precipitation reactions were obtained with sap from plants infected with S.E.V., both in the crude state as expressed through muslin and after clarification by centrifugation. Crude sap usually reacted at dilutions down to 1/16, and sap clarified by the addition of Na_2HPO_4 and centrifugation reacted at 1/4 or 1/8. Preparations of S.E.V. made by precipitation with ammonium sulphate reacted at greater dilutions, sometimes at 1/256 or 1/512. With all the antigen preparations except crude sap, which gives a bulky green precipitate consisting largely of unspecific material, the precipitate has a fluffy open structure similar to that of the antiserum-precipitates formed by bacterial flagellar antigens and by viruses such as tobacco mosaic and potato X, which have asymmetrical particles and show the phenomenon of anisotropy of flow.

S.E.V. crude and clarified sap, and in concentrated preparations, has been tested against antisera to tobacco mosaic virus, potato viruses X and Y, and *Hyoscyamus* virus 3. It failed to precipitate with any in similar conditions to those in which it reacted with its own antiserum. Similarly, preparations of these four viruses failed to precipitate with S.E.V. antiserum while reacting with their homologous antisera. This confirms Chester's (1937) second statement that the etch viruses are a serologically distinct group. We are unable to confirm his further statement that S.E.V. was a poor test antigen whereas M.E.V. was a good one. We made many attempts to obtain precipitin reactions between S.E.V. antiserum and crude and clarified saps from plants infected with M.E.V., but all failed. The most likely cause of this failure seemed to be a too small content of M.E.V. in infective sap to give a visible reaction, so attempts were made to concentrate the virus by precipitations with ammonium sulphate. Although this method had worked well in providing antigen preparations with S.E.V., it failed to give preparations of M.E.V. that would precipitate with S.E.V. antiserum. It then seemed possible that M.E.V. and S.E.V. might be serologically unrelated in spite of the fact that both gave such similar intracellular inclusions, and a rabbit was therefore immunized with clarified sap from plants infected with M.E.V. It was given five intravenous injections of 2 c.c. each and then, as these produced increasingly severe signs of anaphylactic shock, three further intraperitoneal injections of 6 c.c. each. The serum produced in this manner also failed to precipitate with crude or clarified sap from mild etch plants or with preparations made by precipitations with ammonium sulphate. That the serum contained specific antibodies, however, was clearly shown when it was tested against preparations of S.E.V., for then precipitates were obtained exactly as if antiserum prepared against S.E.V. itself had been used. The serum was weaker than those produced against S.E.V. giving a titre of only 1/16. Thus it seems clear that S.E.V. and M.E.V. are serologically related virus strains, that is, they contain common antigenic groupings, for when either is injected into rabbits antibodies are produced with which S.E.V. unites and precipitates. The most probable explana-

tion of their different serological behaviour is a quantitative one; whereas S.E.V. occurs in sufficient concentration in infective sap to precipitate as well as to cause the production of antibodies, the concentration of M.E.V. is sufficient to cause the production of antibodies but not to give a visible precipitate when mixed with them. This view is supported by the fact that comparative infectivity tests between sap from plants infected with S.E.V. and M.E.V., both by local lesions and infection end-points, showed the former to have at least four times the virus content of the latter.

To test further the relationship between M.E.V. and S.E.V., plants infected with the former were reinoculated with S.E.V. Healthy control plants inoculated at the same time developed typical symptoms of severe etch, but none infected with M.E.V. did. These tests were done in the winter, and the protection afforded by the M.E.V. was not complete, for the young actively growing leaves showed a slight increase in mottling, but nothing comparable with the severe disease produced in the plants infected with S.E.V. alone. In experiments done during the summer in better growing conditions protection was more complete, and plants previously suffering from mild etch showed no increase in symptoms when reinoculated with S.E.V. Thus by the plant protection test, as well as by serological methods, S.E.V. and M.E.V. can be classified as related strains. The S.E.V. was obviously able to enter and move through plants already suffering from mild etch, for the young leaves of the reinoculated plants, even when they showed no increase in symptoms, contained this virus, which could be detected by further inoculations to healthy plants. The basis of the protection seems to lie in the presence of the M.E.V. preventing the multiplication of the S.E.V. sufficiently for it to produce its characteristic symptoms. This was indicated by the fact that the virus content of the reinoculated plants did not increase significantly above that of plants infected with M.E.V. alone. It was insufficient to give a precipitate with antiserum and its infectivity, as indicated by starch lesions, was the same as that of normal mild etch sap.

As a further test of possible relationships between S.E.V. and other viruses, plants infected with tobacco mosaic virus, potato viruses *X* and *Y*, and *Hyoscyamus* virus 3 were reinoculated with S.E.V. All of them became fully infected. The external symptoms produced by *Hyoscyamus* virus 3 resemble to some extent those of severe etch, so the appearance of these plants altered only slightly, but the others developed the typical severe etch symptoms. Microscopical examination showed the presence of the characteristic intranuclear inclusions in all four kinds of reinoculated plants. Sap taken from the young leaves of these plants one month after reinoculation reacted with S.E.V. antiserum as strongly as that taken from plants infected with S.E.V. alone, showing that the presence of these viruses, in contrast to M.E.V., had no effect on the multiplication of S.E.V.

An unexpected result was obtained with the plants infected with potato virus *Y* (P.V.Y.) and *Hyoscyamus* virus 3 (HY.V.3). Sap from plants infected with either of these viruses alone reacts with its homologous antiserum at dilutions of from 1/8 to 1/16, indicating that their virus content is much the same as that of severe etch. One month after the plants had been reinoculated with S.E.V., however, sap taken from young leaves failed to react with antiserum to the virus with which the plants were originally infected, i.e. with either HY.V.3 or P.V.Y. antiserum, and reacted only with S.E.V. antiserum. The behaviour of sap from the plants infected with potato virus *X* and then reinoculated with S.E.V. was quite different, for this reacted with both S.E.V. and potato virus *X* antisera; similarly, sap from the reinoculated tobacco mosaic plants reacted with both S.E.V. and tobacco mosaic virus antisera. One

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possible explanation of this result was that HY.V.3 and P.V.Y. were present in the saps of the reinoculated plants but were unable to react with their respective antisera in the presence of S.E.V. To test this, sap was taken from plants infected separately with these two viruses and mixed with sap from either healthy plants or plants infected with S.E.V. These mixtures were then tested serologically. The mixing with healthy sap slowed down the rate of precipitation, but the S.E.V. sap had no additional effect, both HY.V.3 and P.V.Y. reacting with their antisera in its presence. Thus the result with the saps of the reinoculated plants strongly suggested that S.E.V. was able to suppress and replace P.V.Y. and HY.V.3, even in plants in which they had become well established. As such a phenomenon had not previously been recorded, further experiments were made to test the possibility in greater detail.

First, the effect on the virus content of plants by inoculating them with mixtures of viruses was tested. Healthy plants were inoculated with the following seven viruses or mixtures of viruses, the mixtures consisting of equal quantities of crude sap from plants infected with the specified virus: (1) S.E.V., (2) HY.V.3, (3) P.V.Y., (4) S.E.V., P.V.Y., (5) S.E.V., HY.V.3, (6) S.E.V., P.V.Y., HY.V.3, (7) HY.V.3, P.V.Y. The plants rubbed with (1), (4), (5) and (6) all developed typical symptoms of severe etch, those rubbed with (2) and (7) developed symp-

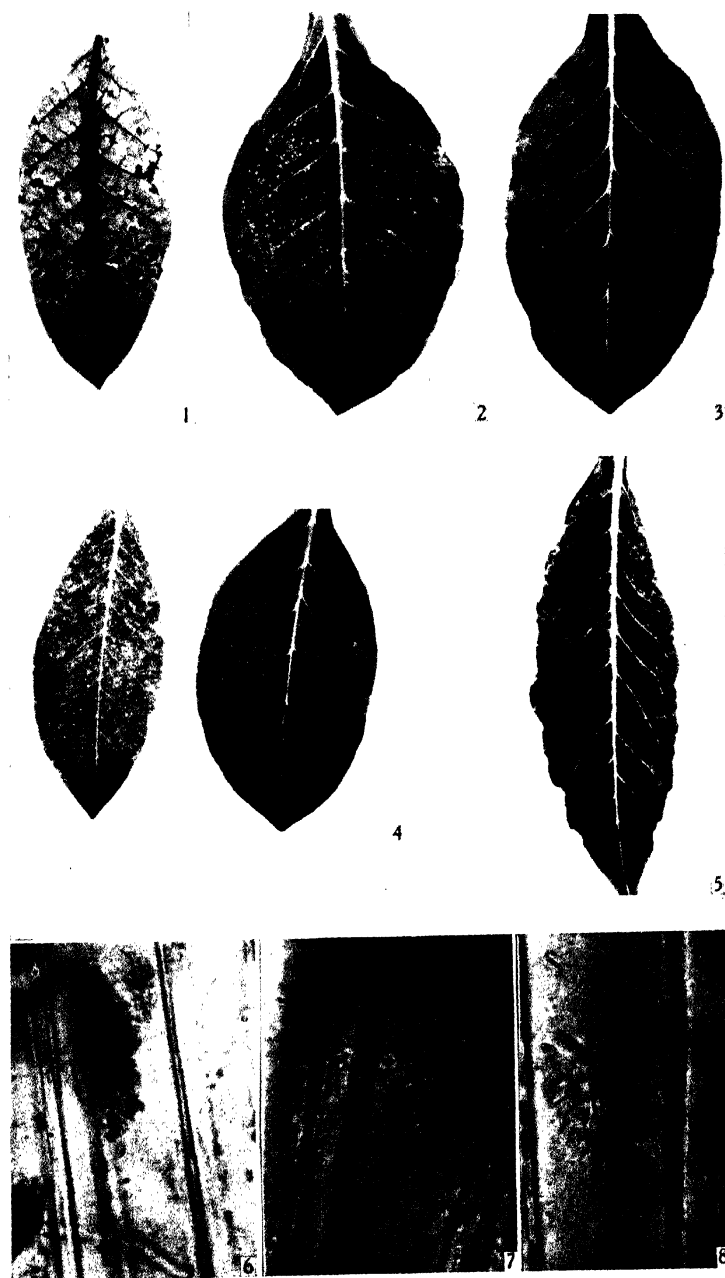
TABLE 1. *Precipitin reactions of saps taken from plants inoculated with severe etch virus, Hyoscyamus virus 3 and potato virus Y alone and in mixtures*

Antigen Sap from plants inoculated with	Antiserum		
	S.E.V.	HY.V.3	P.V.Y.
S.E.V.	+	—	—
HY.V.3	—	+	—
P.V.Y.	—	—	+
S.E.V., P.V.Y.	+	—	—
S.E.V., HY.V.3	+	—	—
S.E.V., HY.V.3, P.V.Y.	+	—	—
HY.V.3, P.V.Y.	—	+	—

+ indicates specific precipitate; — indicates no precipitate.

toms typical of infection with HY.V.3, and only those rubbed with (3) showed the vein-banding characteristic of infection with P.V.Y. Three weeks after infection, sap was expressed from each batch of plants, clarified by a few minutes centrifugation at 16,000 rev./min., and tested against antisera. The summarized results are given in Table 1, and show that the presence of S.E.V. in the inoculum prevented both HY.V.3 and P.V.Y. from multiplying sufficiently to give a precipitin reaction, while the presence of HY.V.3 similarly prevented the multiplication of P.V.Y. Minute amounts of S.E.V. in the inoculum are sufficient to prevent the multiplication of HY.V.3. In one experiment crude sap from severe etch plants was diluted in sap from plants with HY.V.3, and at all dilutions up to 1/1000, i.e. almost the dilution end-point of S.E.V. in water, the plants developed severe etch, and their sap failed to react with antiserum to HY.V.3.

To test the specificity of this reaction tobacco plants were also inoculated with S.E.V., potato virus X, and tobacco mosaic virus, both alone and in mixtures. After 3 weeks, clarified sap from these plants was titrated against antisera to the three viruses (see Table 2). Although the precipitin titres of tobacco mosaic virus and potato virus X are much greater than that of S.E.V., there is no significant difference between the titres of these viruses whether they are in a plant alone or in combination. Similarly, when the sap of plants infected with



both potato viruses *X* and *Y* was titrated against antisera, the titre with virus *Y* antiserum was the same as that of sap from plants infected with virus *Y* alone, and the titre with virus *X* antiserum the same as that given by control plants infected with virus *X* alone.

As all workers may not accept the precipitin titres as an accurate index of the virus content of sap, a further test was done with the mixtures containing tobacco mosaic virus to see if the presence of potato virus *X* or S.E.V. reduced the number of lesions caused by tobacco mosaic virus. Saps taken from plants infected with the virus mixtures were compared, on opposite half-leaves of *Nicotiana glutinosa* at various dilutions, with sap from control plants infected only with tobacco mosaic virus. The results are given in Table 3, and agree with the serological results in showing no significant difference between the content of tobacco mosaic virus in the various plants.

TABLE 2. *Precipitin titres of saps taken from plants inoculated with severe etch virus, potato virus X and tobacco mosaic virus alone and in mixtures*

Antigen	Dilution of sap	Antiserum					
		S.E.V.		P.V.X.			T.M.V.
		1/4	1/8	1/50	1/100	1/200	1/400 1/800
S.E.V.		++	+	—	—	—	—
P.V.X.		—	—	++	+	—	—
T.M.V.		—	—	—	—	—	++ +
S.E.V., P.V.X.		++	+	+++	++	+	—
S.E.V., T.M.V.		++	+	—	—	—	++ +
S.E.V., P.V.X., T.M.V.		++	+	++	+	—	++ +
P.V.X., T.M.V.		—	—	++	+	—	++ +

+ indicates specific precipitates; — indicates no precipitate.

TABLE 3. *Infectivity of saps from plants infected with mixtures of severe etch virus, potato virus X, and tobacco mosaic virus compared with that of sap from plants infected with tobacco mosaic virus alone*

Virus mixture	Average number of lesions per leaf at		
	1/100	1/1000	1/10,000
T.M.V. + S.E.V.	22.7	26.2	1.9
T.M.V.	22.3	25.1	1.8
T.M.V. + P.V.X.	29.3	3.6	0.4
T.M.V.	36.5	4.1	0.5
T.M.V. + P.V.X. + S.E.V.	31.7	4.7	1.3
T.M.V.	46.6	5.9	2.7

Ten half-leaves were inoculated for each test. The numerator is the average number of lesions caused by the virus mixture and the denominator the average number caused by the T.M.V. control on the opposite halves of the same leaves.

From these results it seems clear that it is not a general result for the presence of one virus to restrict the multiplication of another in a plant. The ability of S.E.V. to do this to H.V.3 and P.V.Y., and of H.V.3 to inhibit P.V.Y., suggests some kind of relationship between them. This effect has been confirmed in many separate experiments, both in which plants have been infected with mixed inocula and in which plants previously infected with one or other of these viruses have been reinoculated with S.E.V. The only occasions when S.E.V. has failed to supplant these viruses has been in the winter in bad growing conditions when plants infected with H.V.3, and already in a wilted condition, were reinoculated.

The replacement of P.V.Y. in actively growing leaves by S.E.V. and H.V.3 can be followed

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clearly by testing sap serologically at various intervals after reinoculation. This is shown in Table 4. Plants that had been infected with P.V.Y. for 13 days, when they showed typical symptoms and their sap reacted at 1 in 4 with virus Y antiserum, were divided into three. One set was left as a control, another inoculated with S.E.V., and the third inoculated with H.V.3. At 5, 9, 15 and 21 days samples were taken from young leaves, their saps expressed and tested for their precipitation with antisera (Table 4).

When fairly large plants are infected with S.E.V. only the young, actively growing leaves develop symptoms, the mature leaves remaining apparently healthy and virus-free. Similarly, if plants that have been infected with P.V.Y. for some time are reinoculated with S.E.V. only the young leaves develop symptoms of etch, the old ones retaining the mild vein-banding symptoms. When sap is taken from such plants and tested serologically, that from the upper leaves reacts with S.E.V. antiserum and not with virus Y antiserum, whereas that taken from the older leaves reacts with virus Y antiserum and not S.E.V. antiserum.

TABLE 4. *Change in serological reaction when plants infected with potato virus Y are reinoculated with severe etch virus or Hyoscyamus virus 3*

Dilution of antigen ... Antigen Time		Antiserum											
		P.V.Y.				S.E.V.				H.V.3			
		1/1	1/2	1/4	1/8	1/1	1/2	1/4	1/8	1/1	1/2	1/4	1/8
P.V.Y., control	5	+++	++	+	-	-	-	-	-	-	-	-	-
	9	++++	+++	++	+	-	-	-	-	-	-	-	-
	15	++++	+++	++	+	-	-	-	-	-	-	-	-
	21	++++	+++	++	+	-	-	-	-	-	-	-	-
Reinoculated with S.E.V.	5	+++	++	-	-	-	-	-	-	-	-	-	-
	9	-	-	-	-	-	-	-	-	-	-	-	-
	15	-	-	-	-	++++	+++	++	+	-	-	-	-
	21	-	-	-	-	++++	+++	++	+	-	-	-	-
Reinoculated with H.V.3	5	+++	++	+	-	-	-	-	-	-	-	-	-
	9	+++	++	+	-	-	-	-	-	-	-	-	-
	15	-	-	-	-	-	-	-	-	+++	++	+	-
	21	-	-	-	-	-	-	-	-	++++	+++	++	+

The time is given as days after inoculation. + indicates specific precipitate; - indicates no precipitate.

Whether the presence of S.E.V. suppresses P.V.Y. and H.V.3 completely, or only greatly reduces them, cannot be stated definitely, although the former seems more probable. Sap from reinoculated plants, and from plants inoculated with virus mixtures, has been precipitated with ammonium sulphate, a treatment known to concentrate P.V.Y. and H.V.3 (Bawden & Pirie, 1939), and then tested against antisera. Although this treatment concentrated the S.E.V. eight times, for the precipitin titre went up from 1/8 to 1/64, the preparations still failed to react with antisera to P.V.Y. or H.V.3. Similarly, attempts to demonstrate these two viruses in the young leaves of plants inoculated with them and S.E.V. by means of aphides have failed. A large number of transmissions have been made from such leaves but all of them gave S.E.V. alone. That some infections with P.V.Y. or H.V.3 might have been expected if they were present in these leaves is indicated by the fact that when leaves were rubbed over their whole surfaces with mixtures of S.E.V. and these, P.V.Y. and H.V.3 could frequently be recovered by means of aphides. Thus from plants infected with such mixtures, aphides fed on the inoculated leaves sometimes transmitted S.E.V. and sometimes either P.V.Y. or H.V.3, but from the leaves that became infected by systemic spread they transmitted only S.E.V.

PROPERTIES IN VITRO

In all the tests made S.E.V. behaved very like P.V.Y. and H.V.3.

Dilution end-point. Infections with S.E.V. were rarely obtained at dilutions of crude sap greater than 1/5000, and with M.E.V. the dilution end-point was usually around 1/1000. Stanley & Loring (1938) found that the virus content of Turkish tobacco was nearly twice that of *N. glutinosa*. We have found no such difference between White Burley tobacco and *N. glutinosa*. When saps from plants of these two species, which had been infected for the same length of time, were tested on opposite half-leaves for their infectivity over a range of dilutions they gave no significant differences, and they gave the same precipitation end-point when tested serologically.

Thermal inactivation point. Heating crude S.E.V. sap for 10 min. at 58° C. rendered it non-infective, and heating for this time at temperatures between 52 and 58° C. greatly reduced infectivity. M.E.V. was completely inactivated by 10 min. heating of crude infective sap at 54° C.

Longevity. After 5 days' storage at room temperature the infectivity of S.E.V. sap had fallen to less than 1/100 of the original, but complete inactivation was not obtained until 13 days. In picked leaves left to dry at room temperature, the virus survived a little longer, but was inactive after 24 days. Sap from plants with mild etch was completely inactive after 8 days at room temperature. Loss of infectivity on heating or ageing is a gradual process, and it is to be expected that the time required for complete inactivation will be affected by virus content. It is probable that the differences in the thermal inactivation points and longevity of S.E.V. and M.E.V. are a reflexion of their differing concentrations rather than of differences in stability.

Filterability. S.E.V. in sap clarified by centrifugation passed through a Pasteur-Chamberland L_1 filter candle, but not through L_2 . Attempts to filter the virus through L_3 and L_5 candles were made at various pH values, but all failed, although it was found that adjusting the pH to 7 or higher greatly facilitated the passage of the virus through beds of diatomaceous earth.

Effect of trypsin. When trypsin was added to preparations of S.E.V. their infectivity was immediately reduced, the reduction being proportional to the amount of trypsin added. When incubated with trypsin under conditions in which the enzyme is proteolytically active no further fall in activity occurred. The fall in infectivity on mixing was not reflected by a similar fall in the serological activity, which also remained constant during incubation. Thus S.E.V. behaves towards trypsin like all other plant viruses that have been tested, with the exception of potato virus X (Bawden & Pirie, 1939). The enzyme does not hydrolyse the viruses, but its presence in the inoculum greatly reduces infectivity.

Partial purification. S.E.V. is readily absorbed on to other materials, and any treatment causing a large precipitate in infective sap also causes loss of virus. The precipitates that separate when acid or alcohol is added carry with them all the virus, that caused by freezing carries most of it, and the least loss during clarification of crude sap is produced by the addition of sufficient Na_2HPO_4 to cause flocculation. The instability of S.E.V., coupled with this loss by absorption, makes its rigorous purification extremely difficult by precipitation methods, but partially purified and greatly concentrated preparations can be made by the following method. After clarification by the addition of Na_2HPO_4 and centrifuging, the infective sap is two-fifths saturated with ammonium sulphate. The brown precipitate is spun off, suspended in a volume of water equal to about one-fifth of the original sap, brought to pH 7, and centrifuged to free from insoluble material. The supernatant fluid is one-fifth saturated with ammonium sulphate and centrifuged. The precipitate contains some of the virus, but most remains in the supernatant fluid, which is then two-fifths saturated with ammonium sulphate and again centrifuged. This precipitate is taken up in water, adjusted to about pH 7.8 with alkali, and then incubated for 3 hr. with 0.3 % trypsin. After centrifuging, the preparation is again one-fifth saturated with ammonium sulphate and the precipitate formed removed by centrifugation. The virus is precipitated from the supernatant fluid by the addition of more ammonium sulphate, spun off and dissolved in water. Using this method preparations have been made which showed definite anisotropy of flow, and which reacted with antiserum up to dilutions of 1/512. They were always brown and contained too much impurity for analytical figures to be of any value as an indication of the chemical nature of the virus. However, the preparations behaved so similarly to those of P.V.Y. and H.V.3 (Bawden & Pirie, 1939) that it is reasonable to assume that S.E.V. is also a nucleoprotein with asymmetrical particles. As with these two viruses, it seems that the processes of purification used for S.E.V. lead to a decrease in infectivity without a corresponding loss of serological activity, for these highly active antigenic preparations were always much less infective than crude infective sap. Also, on storage their infectivity rapidly declined whereas their serological activity decreased much more slowly. This

method failed to produce concentrated preparations of M.E.V., probably because of the smaller virus content of sap, which would make the losses by absorption on the discarded precipitates so much more important.

DISCUSSION

The main point arising from this work that calls for discussion is the relationship between the etch viruses and potato virus *Y* and *Hyoscyamus* virus 3. The two etch viruses are clearly strains of the same virus, related to one another in the same manner as are, for example, tobacco mosaic and aucuba mosaic viruses, for they contain common antigens and plants infected with one are protected against infection by the other. The relationship of S.E.V. with P.V.Y. and H.Y.V.3, however, seems to differ from any previously recognized. The three viruses are transmitted by insects in precisely the same manner, falling into the group called 'non-persistent' by Watson & Roberts (1939), and have essentially similar physico-chemical properties. Yet they are not serologically related and plants infected with P.V.Y. or H.Y.V.3 are not protected against S.E.V. They cannot, therefore, be regarded as related strains in the sense in which the phrase is at present applied to viruses. However, the fact that plants infected with S.E.V. are protected against infection with P.V.Y. or H.Y.V.3, and plants with H.Y.V.3 are protected against P.V.Y. suggests some fairly close relationship between the three, for when any of these is transmitted to plants already infected with a virus with quite dissimilar properties, for example, potato virus *X*, it enters and multiplies as in healthy plants. If words coined for use with higher organisms can legitimately be applied to viruses, M.E.V. and S.E.V. can probably be regarded as recently derived varieties of a single species, and the etch viruses, P.V.Y. and H.Y.V.3 as separate species of the same genus. It is possible that other viruses of the non-persistent group, e.g. cucumber virus 1 and potato virus *A*, which also have similar properties *in vitro*, will be found to be related to S.E.V., P.V.Y. and H.Y.V.3 in the same manner.

In previous work on the ability of one virus to protect plants against infection with others, serological methods could not be used, for only serologically related viruses were known to interact in this manner. The work mainly consisted in demonstrating that a virus causing a mild disease protected plants against serologically related viruses causing more severe symptoms, although that the protection is reciprocal has occasionally been shown (Bawden, 1939). If either one of a pair of such related viruses is established in a plant the other is unable to develop, but if the two are inoculated together to a healthy plant both usually enter and multiply, the resulting symptoms being a compromise between those caused by the two viruses acting alone. The interactions of S.E.V. and M.E.V. provide another illustration of this well-known phenomenon. The interactions of S.E.V., P.V.Y. and H.Y.V.3 differ from those of virus strains in three ways. First, the protection is not reciprocal, for although plants already infected with S.E.V. are protected against the other two, plants infected with these are not protected against the more severe disease caused by S.E.V. Secondly, when these viruses are introduced into plants together with S.E.V. they are unable to multiply detectably and the resulting symptoms are those of severe etch. Thirdly, even in plants in which they are well established they are suppressed and replaced by S.E.V. The mechanism underlying the protection afforded to a plant against one strain by previous infection with another is not yet established, but it is generally believed to be because the strains either utilize the same materials or the same sites for reproduction, and if one is already fully established the other is unable to multiply. Two possible explanations offer themselves for

the different interactions between S.E.V., P.V.Y. and HY.V.3. Infection with S.E.V. may give rise to conditions in the plant that inactivate the other two viruses, although the viruses have such similar stabilities in vitro that it is difficult to imagine conditions which would inactivate these without also inactivating S.E.V. Alternatively, these three viruses may be as unstable and have as short an existence in vivo as in vitro, and in the plant they may be continually being destroyed, the content of active virus being kept reasonably constant by the continual production of new virus. Then, if these viruses are produced from the same materials or at the same sites, but have different affinities for these materials or sites, with S.E.V. having the greatest and P.V.Y. the least affinity, these results can be interpreted. For when plants are inoculated with mixtures of the viruses, the constituent with greatest affinity will multiply more rapidly and usurp materials or sites needed by the others; and when plants already infected with a virus of small affinity are reinoculated with one of greater affinity, the original infecting virus will be replaced by the second as it becomes inactivated. The differences between the reciprocal protection afforded by strains of one virus and the interactions between these three serologically unrelated viruses can be interpreted on the basis that strains both utilize and have the same affinities for the same 'virus-making' sites or materials, whereas these viruses utilize the same materials but have different affinities for them.

Although no appreciable differences have been found between the properties of S.E.V., P.V.Y. and HY.V.3 in vitro, they differ widely in their behaviour in the plant. S.E.V. causes the production of large numbers of inclusions in the nuclei and in the cytoplasm; HY.V.3 causes large numbers of cytoplasmic inclusions, but none has been found in plants infected with P.V.Y. Bawden & Sheffield (1939) suggested that the inclusions formed by tobacco mosaic and related viruses might be insoluble complexes formed by the union of the viruses with host constituents. They could not suggest whether these constituents were normal plant products or abnormal ones produced as a result of virus activity. The three insect-transmitted viruses precipitate in such similar conditions in vitro that it is to be expected that any normal plant constituent which precipitates one would also precipitate the others. Therefore, if the view be adopted that the inclusions formed by S.E.V. and HY.V.3 are also insoluble virus-host complexes (a view supported by Sheffield's (1941) discovery that the inclusions are infective and chemically complex), it seems probable that the substances with which the viruses unite are not normal host constituents, but products specific to the particular infection. The different external symptoms caused by the three viruses show that they have different physiological activities and attack different materials in the plant; the different cytological pictures may be a further indication of these differences.

SUMMARY

The symptoms of severe and mild etch are described; plants suffering from either contain both intranuclear and cytoplasmic inclusions. Fewer and larger crystals are formed in the nuclei of plants with mild etch. Plants infected as seedlings with S.E.V. develop malformed leaves, in which the cytoplasmic inclusions crystallize to give rise to birefringent needles. M.E.V. protects plants against infection with S.E.V.; the two are serologically related, for antisera prepared against either reacted with S.E.V. Precipitation was not obtained with M.E.V., presumably because the virus content of sap is too small.

Although not serologically related to potato virus Y or Hyoscyamus virus 3, S.E.V. has similar properties in vitro and is transmitted in the same way. The interactions of these

three viruses in the plant suggests that they are related. Plants infected with either of the other viruses are not protected against S.E.V., and those infected with P.V.Y. are susceptible to H.V.3. Plants infected with S.E.V., however, are protected against the other viruses, and those infected with H.V.3 are protected against P.V.Y. S.E.V. is able to suppress these two viruses when healthy plants are infected with a mixed inoculum, and to supplant them in tissues in which they are already established. Similarly, H.V.3 can suppress and supplant P.V.Y. Possible interpretations are given for these results. S.E.V. has asymmetrical particles, for concentrated preparations showed anisotropy of flow.

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EXPLANATION OF PLATE 6

- Fig. 1. Tobacco leaf inoculated with S.E.V., decolorized and stained with iodine. Before treatment the leaf showed no sign of local lesions.
- Fig. 2. Tobacco leaf inoculated with S.E.V. showing local lesions of the necrotic ring type.
- Fig. 3. Tobacco leaf inoculated with S.E.V. showing diffuse chlorotic local lesions.
- Fig. 4. Young leaves of plants recently infected with S.E.V., showing vein clearing and necrotic etching. Note the symptoms spreading from the bases to the tips.
- Fig. 5. Leaf from a plant infected with S.E.V. for a month showing a general yellow mottling.
- Fig. 6. Epidermal cell from tobacco leaf infected with M.E.V., fixed in formol saline and stained with haematoxylin, showing a nucleus containing a single large crystal and the nucleolus. $\times 900$.
- Fig. 7. Living epidermal cell from tobacco plant infected with M.E.V. showing a crystal with a definite cross suggesting a bi-pyramid shape. $\times 900$.
- Fig. 8. Epidermal living cell from a malformed leaf of a tobacco plant infected with S.E.V. when a seedling. The cytoplasmic inclusion has crystallized and contains needles that are birefringent. $\times 900$.

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TRANSMISSION OF TOBACCO ETCH VIRUSES BY APHIDES

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THE fact that severe etch virus (S.E.V.) is transmitted by *Myzus persicae* Sulz. has already been recorded (Kassanis, 1939). This paper gives the results of more detailed studies on the relationships between tobacco etch viruses and their vectors.

Most of the work was done with S.E.V. obtained from Dr W.M. Stanley, but comparative tests were made with the related mild etch virus (M.E.V.) supplied by Dr W.C. Price. The conditions of the insectary and methods of handling and culturing the aphides were similar to those described by Watson (1936, 1938). The aphides were raised on turnips and radishes, both of which are immune to the etch viruses. Leaves of medium size, cut from tobacco plants infected for at least 14 days, were used for the infective feeding, and young tobacco seedlings were used as test plants. The first symptom appears 6-8 days after infection and usually not on all plants in the same day. The time probably depends on the part of the leaf in which the insect was feeding, so that the virus has to travel different distances to reach the central vein. Examination of the leaves infected by single aphides, by the iodine method, shows a retention of starch at the point of infection.

Preliminary tests showed that aphides, fed for only 2 min. on an infected leaf, and transferred immediately to test plants, could infect them even if removed after 2 min. Thus the whole process of acquiring the virus and infecting a healthy plant can occur within 5 min., showing that there is no delay in transmission such as occurs with some viruses. These tests also showed that infection could be obtained readily with a single aphid, and in all the experiments described, unless otherwise stated, only one aphid per plant was used. The phrase 'percentage of infection' is the number of plants out of 100 infected by single aphides.

In addition to *M. persicae* it was found that S.E.V. is transmitted by *M. circumflexus* (Buckt.), *Aphis rhamni* (Boyer), *Aphis fabae* (Scop.) and *Macrosiphum gei* (Koch.).

EXPERIMENTAL

Effect of variations in the length of preliminary starving and infective feeding times

Watson (1938) showed that the percentage of infection with Hyoscyamus virus 3 was greatly increased if aphides were starved before getting their infective feeding, provided the time of infective feeding was also short. Similar results have been obtained with both S.E.V. and M.E.V. Table 1 shows the summarized results of 11 experiments with S.E.V. In each experiment forty-five plants were used, five each for nine different treatments. The treatments consisted of variations of from 0 to 4 hr. in the starving time before infective feeding and from 2 min. to 1 hr. for the infective feeding time.

Increasing the preliminary starving time increases the percentage of infection. This effect disappears if the infective feeding time is itself increased, so that with an infective feeding time of 1 hr. the previously starved insects transmit no better than those receiving no preliminary starving period. The longer the time that aphides fed on the source of the virus the less likely were they to transmit, and when aphides that had been raised on infected tobacco plants were transferred to healthy plants the percentage of infection was very small. The most infections with single aphides occur when they have not fed for at least 4 hr. and are then fed for only a few minutes on the infected plant.

Table 2 shows the summarized results of four replications of an experiment made to compare the transmission of M.E.V. by *M. persicae* with that of S.E.V. There were six treatments for each virus, and a total of thirty-five aphides was used for each treatment. The treatments consisted of a preliminary starving period of either 0 or 4 hr., combined with an infective feeding time of 2 min., 1 hr. or 4 hr.

M.E.V. reacts to variations in preliminary treatment and in the length of the infective feeding exactly like S.E.V., and the two viruses are equally readily transmitted. This differs from results obtained by Watson (1939) with mild and severe strains of *Hyoscyamus virus 3*. The severe strain gave a higher percentage of infection, which Watson correlated with the higher virus content of sap infected with this strain. The virus content of sap from plants with S.E.V. is also considerably higher than that from plants with M.E.V. (Bawden & Kassanis, 1941), yet these two are transmitted to the same extent.

TABLE 1. *Effect of variations in the length of preliminary starving and infective feeding times*

Preliminary starving time	Infective feeding time			Total	% of infection
	2 min.	15 min.	1 hr.		
None	6	5	1	12	7.2
1 hr.	22	11	3	36	21.8
4 hr.	32	7	1	40	24.2
Total	60	23	5	88	
% of infection	36.3	13.9	3.0		

TABLE 2. *Comparative transmission of S.E.V. and M.E.V.*

Preliminary starving time	Strain of virus	Infective feeding time			Total	% of infection
		2 min.	1 hr.	2 hr.		
None	S.E.V.	2	0	—	2	5
	M.E.V.	3	0	—	3	8
4 hr.	S.E.V.	16	0	0	16	45
	M.E.V.	15	1	0	16	45

TABLE 3. *Effect of post-infection starving time*

Preliminary starving time	Post-infection starving time				
	None	15 min.	1 hr.	3 hr.	6 hr.
None	14 %	0	0	0	0
4 hr.	52 %	49 %	34 %	17 %	0

Effect of variations in treatment after the infective feeding

Preliminary tests showed that not only was no incubation period necessary before the aphides could transmit S.E.V. but that they failed to transmit unless they were placed on test plants soon after removal from the source of infection. Table 3 shows the summarized results of ten replications of an experiment made to determine how long *M. persicae* retained its infectivity after removal from the source of infection. In each replication fifty plants were used, five for each of the ten treatments. The treatments consisted of preliminary starving of either 0 or 4 hr., and variations in the period before being placed on the test plants of from 0 to 6 hr. All the aphides received an infective feeding period of 2 min.

The results again show the effect of preliminary starving in increasing the percentage of infection, and also that the insects treated in this way retain their infectivity longer. Even these, however, had completely lost their ability to infect healthy plants after 3 hr.

It seemed possible that temperature might affect the length of time for which the aphides remained infective, and a comparison was therefore made with aphides kept at 3° C. after being fed for 2 min. on the infective plant. This experiment was repeated eight times. On each occasion 5 plants were used for each treatment, and the percentage of infection obtained is given in Table 4. These results can be contrasted with those of Table 3 obtained at a temperature of about 20° C.

Here again the beneficial effects of a preliminary starving in increasing the length of time for which the aphides remain infective are shown, and also those of lowering the temperature. The effect of temperature may partly explain the variation in percentage of infection obtained at different seasons. Aphides, under optimal conditions for transmission, in the months of June–August gave 46 % whereas in the colder months of October–December they gave 59 %, although growing conditions for both plants and insects were less favourable.

TABLE 4. *Effect of post-infection starving time at 3° C.*

Preliminary starving time	Post-infection starving time								
	None	15 min.	1 hr.	2 hr.	3 hr.	4 hr.	16 hr.	20 hr.	24 hr.
None	12 %	15 %	10 %	2 %	5 %	—	—	—	—
4 hr.	—	37 %	—	—	—	22 %	20 %	10 %	7 %

Watson & Roberts (1939) have shown that individuals of *M. persicae* remain infective with Hyoscyamus virus 3 longer if they are starved after their infective feeding than if they feed continuously. Aphides infective with S.E.V. behave similarly. Eight replications of an experiment were made in which insects, first starved for 4 hr. and then given an infective feeding period of 2 min., were allowed to feed for varying periods on a healthy plant before being transferred to the test seedlings. The percentage of infection obtained with insects left on the healthy plant for 2, 5, 15 and 30 min. was 35, 35, 2 and 0 % respectively. Thus, in contrast to the 3 hr. for which insects starved at room temperature remained infective, those feeding continuously lost most of their infectivity in 15 min. and all of it in 30 min.

As the insects lose their infectivity so quickly, it is obvious that in natural conditions it must be unusual for one to infect more than one plant. To determine whether insects could infect more than one plant without further access to a source of infection, the following experiment was repeated three times, using 10 aphides each time. The aphides were given a preliminary starving period of 4 hr., fed for 2 min. on the source of infection, and then transferred to the first series of test plants. After 2–3 min. they were transferred to the second series of plants on which they were also allowed to feed for 2–3 min. They were given similar short feeding periods on a third and fourth series of plants and left overnight on a fifth. Seven of the aphides failed to infect any of the test plants and the performances of the other twenty-three are recorded individually in Table 5, infected tests plants being shown by a +.

Only one aphid succeeded in infecting four plants and two aphides infected three plants. More infections occurred in the first set of test plants than in any other, but the total obtained

in the last four sets was greater than that in the first. One of the most striking results is that an aphid may fail to infect from one to four test plants on which it feeds and then infect the next.

It seemed possible that aphides which had once become infective and then lost their infectivity might behave differently from other insects. To test this possibility the same aphides were used in an identical experiment done on consecutive days. Five aphides were used in each experiment and the experiment was repeated five times. The aphides were starved for 4 hr., fed for 2 min. on an infective plant and then transferred singly to healthy test plants, on which they were allowed to remain overnight. The insects were then again starved, given an infective feeding of 2 min. and transferred to the second test plants.

TABLE 5. *Consecutive feeding on a series of five plants*

Consecutive healthy plants					Total of infected plants
A	B	C	D	E	
o	+	o	o	o	1
+	o	o	o	o	1
o	+	o	+	o	2
o	+	o	o	+	2
o	o	o	o	+	1
+	o	o	o	+	2
+	+	o	o	o	2
+	o	o	o	+	2
+	o	o	o	+	2
+	+	+	o	o	3
o	+	o	o	+	2
+	o	o	o	o	1
+	o	+	o	o	2
+	o	o	o	+	2
+	o	o	o	o	1
+	o	o	o	o	1
+	o	o	o	o	1
+	o	+	o	o	2
+	+	+	o	o	3
+	o	o	o	o	1
+	+	o	+	+	4
Total 18	8	5	2	8	41

The total infections obtained on the first days were nineteen and on the second twenty-four. The records of the individual aphides showed that some transmitted on the first day but not on the second, some on the second but not on the first, and some on both days. The fact that an aphid transmitted on one day but not on another suggests that the failure to get 100 % infection in transmission tests with single aphides is not because the culture of aphides used contains individuals unable to transmit, but because of some other unknown factor. The fact that some aphides transmitted on both days also shows that having once been infective has no effect on future ability to transmit.

Transmission from virus mixtures

Aphides, given varying lengths of preliminary starving and infective feeding on plants infected with S.E.V. and tobacco mosaic virus, when transferred to healthy test plants have always no infections or infections with S.E.V. alone.

Bawden & Kassanis (1941) showed that when S.E.V. is inoculated to tobacco plants with *Hyoscyamus virus 3*, the latter appears to be suppressed and replaced by S.E.V., for sap taken from young leaves of such plants reacts only with antiserum to S.E.V. Similarly, the young leaves of plants inoculated with a mixture of the two viruses react serologically only with S.E.V. antiserum. In an attempt to get a more sensitive test for *Hyoscyamus virus 3* than the serological one, aphides were fed on plants rubbed with a mixed inoculum, and then transferred to healthy tobacco plants. Of the 130 test plants, ten developed symptoms typical of infection with *Hyoscyamus virus 3* and thirty-three those of S.E.V. In this experiment no distinction was made between aphides fed on leaves actually rubbed with the inoculum and those fed on leaves with systemic infection. A second test was therefore made with 200 aphides, 100 of which were fed on leaves rubbed with a mixed inoculum of S.E.V. and *Hyoscyamus virus 3* and the other 100 on young leaves of these plants showing systemic symptoms. The results, given in Table 6, show that the aphides were able to recover *Hyoscyamus virus 3* alone from the rubbed leaves but not from the others. It seems that in the rubbed leaves both viruses multiply, but that in those that become infected by systemic spread S.E.V. suppresses *Hyoscyamus virus 3* almost completely. From leaves infected systemically with *Hyoscyamus virus 3* alone, at least 50 plants would have been

TABLE 6. *Transmission from plants inoculated with S.E.V. and Hyoscyamus virus 3*

	Insects fed on rubbed leaves	Insects fed on leaves with systemic infection
Insects infected with <i>Hyoscyamus virus 3</i>	16	0
Insects infected with S.E.V.	39	49
Total	55	49

infected by the aphides under the conditions of the experiment, and the fact that from these leaves none was infected is strong evidence that they contained little or no *Hyoscyamus virus 3*.

DISCUSSION

The results obtained with S.E.V. are very similar to those obtained by Watson & Roberts (1939) with *Hyoscyamus virus 3*, potato virus Y and cucumber virus I. The percentage of infection with all is increased greatly by starving insects before feeding them on the source of infection and is greatly reduced if the period of feeding on the source of infection is increased. The only difference in the behaviour of S.E.V. and *Hyoscyamus virus 3* is that aphides lose their infectivity even more rapidly with S.E.V. than with the latter, and consequently fewer plants can be infected in succession by one aphid. As with *Hyoscyamus virus 3*, the time for which aphides remain infective is greater when they are fasting than when they are feeding.

Some workers, e.g. Doolittle & Walker (1928), suggest that vectors which rapidly lose their infectivity act purely mechanically, their stylets merely behaving as needles which become contaminated with virus while feeding on infected plants. The results described here, however, are difficult to fit to this hypothesis. If it were true, it would hardly be expected that aphides fed on plants infected with both S.E.V. and tobacco mosaic virus would regularly transmit only S.E.V., for the concentration of tobacco mosaic virus in the

sap is at least 100 times that of S.E.V., and it is transmitted mechanically much more easily. Nor would it be expected that infective insects fed successively on a series of healthy plants could fail to infect early ones and then infect later ones in the series. This fact suggests that the aphid is ejecting virus discontinuously and not merely having it absorbed by the plant from the outside of the stylets. Again, the theory of mechanical transmission cannot explain the great increase in the percentage of infectivity obtained by using starved insects given a short infective feeding.

The results with S.E.V. support the suggestion of Watson (1938) that the aphides ingest the virus and that in the aphid the virus comes into contact with something that inactivates it. Her earlier suggestion (1936) that the inactivation resulted from specific antibody formation seems unlikely, for inactivation with S.E.V. would seem to occur too quickly for antibody formation to be possible, and the fact that aphides which have lost their infectivity are as active as vectors as other aphides also tells against the view that they acquire any antibodies against the virus. Her later suggestion that the virus is inactivated by something that is secreted in greater quantity by aphides while feeding than while fasting, e.g. a proteolytic enzyme, would explain all the observed effects with S.E.V.

SUMMARY

Severe etch virus is transmitted by *Myzus persicae*, *M. circumflexus*, *Aphis rhamni*, *A. fabae* and *M. gei*. Although the content of mild etch virus in sap is much less than that of S.E.V., both are transmitted to the same extent by *M. persicae*. The percentage of infection using single aphides is greatest with aphides that are starved for 4 hr. or more and then fed on the source of infection for 2 min. Continuous feeding on healthy plants or diseased plants greatly reduces the efficiency of the vector. The length of time for which aphides remain infective is also increased from 15 min. to a few hours if the aphides are starved instead of allowed to feed; it is also greatly increased in starved insects if they are kept at low temperature. Provided the feeding time on each test plant is small, one aphid may infect up to four plants.

I wish to thank Mrs M. A. Watson for much helpful advice.

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VARIATIONS IN THE CYTOPLASMIC INCLUSIONS INDUCED BY THREE STRAINS OF TOBACCO MOSAIC VIRUS

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(With Plates 13 and 14)

INTRACELLULAR inclusion bodies have been found in only about twenty of the hundred or so plant virus diseases which have been described in detail, but in these they have often proved a useful aid in diagnosis and differentiation. If a virus induces them in one host in which it causes mosaic symptoms, it usually induces their formation in all those hosts which show such symptoms. They do not usually occur in plants showing necrotic symptoms or in carriers. The inclusions of different viruses often differ in morphology and show slight differences in physical and chemical properties, but those induced by any one virus have been fairly constant in form, subject only to slight modification by the host plant and to variation in quantity and distribution. However, in the summer of 1940, several new types of cytoplasmic inclusions were found in plants infected with each of three strains of tobacco mosaic virus: the ordinary tobacco mosaic (Johnson No. 1), aucuba mosaic and enation mosaic viruses.

In 1903, Iwanowski first described the inclusions in tobacco plants with mosaic. He described the amoeba-like bodies, of $5-10\mu$ diameter, which Goldstein (1926) later called *X*-bodies, and also the large plate-like crystals which become striated on treatment with dilute acids. Over a period of nearly 40 years this virus has been worked with, and its effects described by, many workers under varying conditions in several parts of the world. The phenomena observed have been interpreted in various ways but surprisingly few discrepancies exist in the accounts. Casual reference has sometimes been made to the occurrence of a few raphides in infected plants but otherwise descriptions have differed from Iwanowski's only in giving greater detail.

Enation mosaic virus has been less extensively studied. The intracellular inclusions when first examined at Rothamsted in 1936 were indistinguishable from those of tobacco mosaic virus, consisting of an amorphous protein *X*-body and of larger hexagonal protein crystals which were often fused side by side (Sheffield, 1939*b*; Bawden & Sheffield, 1939). This strain was carried on continuously through a series of hosts. Plants examined from time to time showed no variation until the summer of 1940.

Aucuba mosaic virus had shown some slight variation before 1940. It differed from the other two strains in that instead of the amoeboid body, it produced large inclusions of sometimes 30μ diameter by the aggregation and fusion of particles of amorphous protein material, which were precipitated in the streaming cytoplasm. After some weeks crystals were formed within this body. Under some conditions, the amorphous body was not formed but crystals were formed directly. With the amorphous body was often found a long spike-like inclusion (Henderson Smith, 1930; Sheffield, 1931). This inclusion was present

in 1928 and occurred regularly in 1929 and 1930. For four years no records were made but in 1935 its disappearance was commented on and from that time, although it was frequently sought, it was not again observed. The appearance of such spike-like bodies in very large numbers in a plant infected with tobacco mosaic virus led to the discovery of a large number of variations in the cytology of plants infected with any one of the three strains. This paper describes and discusses the new forms.

MATERIAL

When, early in September 1940, the plant suffering from tobacco mosaic was found not to be behaving typically, all other plants in the glasshouses infected with any of the three strains of virus were immediately examined. These, also, all contained atypical inclusions. Inoculations were then made to seedlings of tobacco, tomato and *Solanum nodiflorum* from these plants and also from dried, or purified, specimens of virus. The tobacco mosaic virus used came originally from Dr James Johnson, some had been carried on at Cambridge and some at Rothamsted for many years. Samples of both had been dried or purified at various times over a period of years. The aucuba mosaic virus came originally from Dr W. F. Bewley and had been maintained in the Rothamsted glasshouses for 15 years. Specimens had been dried at a time when spikes were known to be present and also dried or purified when they were known to be absent. The enation mosaic virus was obtained from Dr G. C. Ainsworth in 1936 and has since been carried on in the Rothamsted glasshouses. Some of the inocula were kindly supplied by Mr F. C. Bawden and by Dr J. Henderson Smith. In all, over a dozen sources of virus were used. One or more sets of inoculations were made from each, a minimum of three plants being used for each inoculation. As the results obtained appeared to be independent of the sources of the inocula these are not given in detail.

Most plants were kept in the glasshouse chambers, no attempt being made to control the amount of light or heat available. Early in October a few plants were kept shaded. Tobaccos were infected with tobacco mosaic virus and tomatoes with aucuba mosaic virus. Some of each group put in slight shade became etiolated, and some in dense shade became very stunted.

VARIATIONS

Crystalline inclusions

Hexagonal crystals (Purdy Beale, 1937; Bawden & Sheffield, 1939) were produced by all three strains in all hosts and were found at some period in every plant examined. In tobacco and enation mosaics they appeared soon after the external symptoms. In the plants inoculated in May and June with aucuba mosaic virus their appearance was long delayed as amorphous bodies were first formed and crystals were derived only from these (Pl. 14, fig. 1a-c). In plants inoculated on 19 June and 4 Sept. the bodies began to crystallize in November. Plants inoculated with aucuba mosaic virus in October produced no amorphous bodies but crystals were present in November although no external symptoms were apparent. When plants were shaded from the time of inoculation with aucuba mosaic virus no amorphous bodies were formed but crystalline material appeared after about 7 weeks. Shaded plants inoculated with tobacco mosaic virus produced both amoeboid bodies and hexagonal crystals but not until 7 weeks after infection. The hexagonal crystals are striate in edge view but this is usually seen only through crossed Nicol prisms or on acidification. Occasionally the striations are very conspicuous even in crystals in untreated cells (Pl. 13, fig. 5). This occurs usually in cells which in summer produced the more usual types of inclusions and in winter were forming rather unusual amorphous inclusions.

Fibrous inclusions

Spike-like bodies. These fine needle-shaped bodies had occurred regularly in plants infected with aucuba mosaic virus but had disappeared for some years. Raphides which have occasionally been mentioned in descriptions of tobacco mosaic disease were probably similar; these had never been found at Rothamsted.

Early in September 1940 spike-like bodies were found in all the hosts which had been inoculated in May, June and July with any of the three virus strains. In *S. nodiflorum* infected with aucuba mosaic virus they were about as abundant as in 1929, usually one, occasionally more, occurring in

most of the hair cells and in every cell over large areas of the epidermis. In tobacco infected with tobacco mosaic virus they were very abundant, several being present in almost every epidermal cell (Pl. 13, fig. 1). They appeared first in the hairs and in the epidermis a few days later. On 9 Sept. inoculations were made of all three strains, each obtained from several sources, to a large batch of tobacco plants. Spike-like bodies appeared in all those plants which developed systemic symptoms. Some of these plants were destroyed on 21 Oct.: others kept until the end of November ceased to produce this form of inclusion, which could then be found only in the older leaves. A few were found in *S. nodiflorum* inoculated on 19 Sept. with aucuba mosaic virus but otherwise none was found in plants inoculated later than 9 Sept. although they were present in all plants inoculated between May and that date. In November they were found to be disappearing even from the older leaves of plants in which they had been abundant.

These inclusions were fine needle-shaped bodies with pointed ends and no facets. In length they are usually approximately equal to the longest dimension of the containing cell. Henderson Smith (1930) said that the spike 'can sometimes be seen to be made up of a bunch of hair-like crystals, especially distinguishable at the ends'. Sheffield, who has worked with this virus since 1929, failed to observe this condition until these bodies reappeared in 1940. Careful examination between crossed Nicol prisms often revealed that the body consisted of a number of extremely delicate fibres lying parallel or twisted together. Slight acidification caused these fibres to separate and to become easily visible by transmitted light. Further acidification caused their dissolution. They were weakly birefringent (Pl. 13, fig. 8), of the same sign as the cell wall, the refractive index being higher in the direction of the length of the fibre. They could be fixed in formol-saline or in saturated aqueous picric acid.

Spindle-shaped bodies. Usually only one spindle-shaped body was found in a cell (Pl. 13, fig. 7). It might be about the length of the cell or, if in a very long hair cell, might lie diagonally across one half of it. They were 10–15 μ wide at the centre tapering to extremely fine ends which might be curved. They consisted of aggregates of long fine fibres and might well have been composed of a number of the spike-like inclusions of various lengths packed closely side by side. When viewed between crossed Nicol prisms they were doubly refractive and their fibrous structure became more obvious. They were induced by all three virus strains but were not found in *S. nodiflorum*. They were seen in plants infected in summer but not in those inoculated later than 9 Sept.

Masses of small needle-like fibres. With all three virus strains were found large numbers of fine needle-shaped bodies (Pl. 13, fig. 4). In shape, they resembled the spike-like bodies but were very much finer and shorter: usually their length was less than the width of a hair cell. When seen between crossed Nicol prisms they were weakly birefringent, the higher index being in the direction of the length. In size and appearance they were identical with the fibres formed by the acidification of the hexagonal crystals. Their position often suggested that they might be derived from these. The pH of sap extracted from cells containing them was about 5.6 which is insufficiently acid to produce needle-like forms experimentally from striate material. They also resembled the birefringent para-crystals produced by precipitating the virus with acid below pH 4 or by addition of large quantities of salts (Stanley, 1936; Bawden & Pirie, 1937). The pH of the cell sap is too high and the salt content insufficient to cause the separation of these para-crystals in the living cell. It is known that the masses of needle-shaped bodies were sometimes derived from amorphous bodies of the aucuba type (Pl. 14, fig. 2a) and also from a form of amorphous inclusion which will be dealt with later. Possibly they were also precipitated directly from the cell sap. Of this, it would be difficult to obtain conclusive evidence. They occurred most abundantly in summer but were found occasionally in winter in the older leaves of tomato plants inoculated in early summer. They were not found in *S. nodiflorum*.

Long curved fibres. Greatly elongated fibres were also found either alone in a cell or in association with any of the other forms of inclusion. Structurally they seemed to be similar to the spike-like forms but were several times as long as the cell, being curved and twisted within it. Often they were bent into the form of a figure 8 (Pl. 13, fig. 6). Some in long hair cells were calculated to be as much as 400 μ in length. Circles and tightly coiled fibres wound in the form of a sphere were occasionally found. Like other fibrous forms they were birefringent. They may be similar to the 'rings and figure 8's' described by Soukoff & Vovk (1938) as occurring in a mosaic disease of oats. They may be comparable to the mesomorphic fibres of as much as 2.5 mm. in length which separate from clarified sap from infected tobaccos standing at 1° C. for several months (Best, 1937). These fibres were produced by all three strains. They seemed to occur most abundantly in tomato and were never found in *S. nodiflorum*. They were most abundant in summer but still persisted in winter in some of the older leaves of tomato plants which had been inoculated in early summer.

Amorphous bodies

Several different types of amorphous inclusion occurred. The amoeboid type characteristic of tobacco and enation mosaics was found in all plants infected with these two viruses. It is difficult to make a quantitative estimate but the general impression was that they were seen much less frequently than in plants previously examined. These bodies had never been found in plants infected with aucuba mosaic virus until 1940 when similar structures occasionally appeared (Pl. 14, fig. 6).

The large granular amorphous bodies characteristic of aucuba mosaic were formed in all plants showing systemic infection and inoculated before the end of September. Plants inoculated after that date produced no amorphous inclusions but gave striate material directly. These amorphous bodies, if viewed between crossed Nicol prisms soon after formation, are not birefringent but after some weeks they come to contain a large amount of doubly refractive material (Pl. 14, fig. 1a-c): some of this usually takes the form of hexagonal crystals. In the summer of 1940, the bodies came to contain very large numbers of the small needle-like fibres often to the exclusion of all striate material (Pl. 14, fig. 2a). All stages could be seen between bodies containing only amorphous material and those which were almost completely translated to needle-like fibres. Some amorphous material is always left; presumably it contains the chondriosomes and other substances known to be present in the amorphous bodies. In summer one amorphous body might give rise to both striate material and fibres but towards autumn usually striate material only was formed. The contents of such bodies are in motion: sometimes it is so slow as to be visible only after several hours' observation but at others it is very rapid. In the latter case it usually occurs in loosely packed bodies containing small particles. Sometimes accompanying such disintegrating bodies of this type are found large numbers of smaller bodies bearing some resemblance to the amoeboid bodies of tobacco mosaic. They take the form of hyaline vesicles, usually spherical, and are not more than 10μ in diameter. Each contains several highly refractive granules. These bodies either float in the cell sap or are carried rapidly in the cytoplasmic stream (Pl. 14, fig. 2a-b).

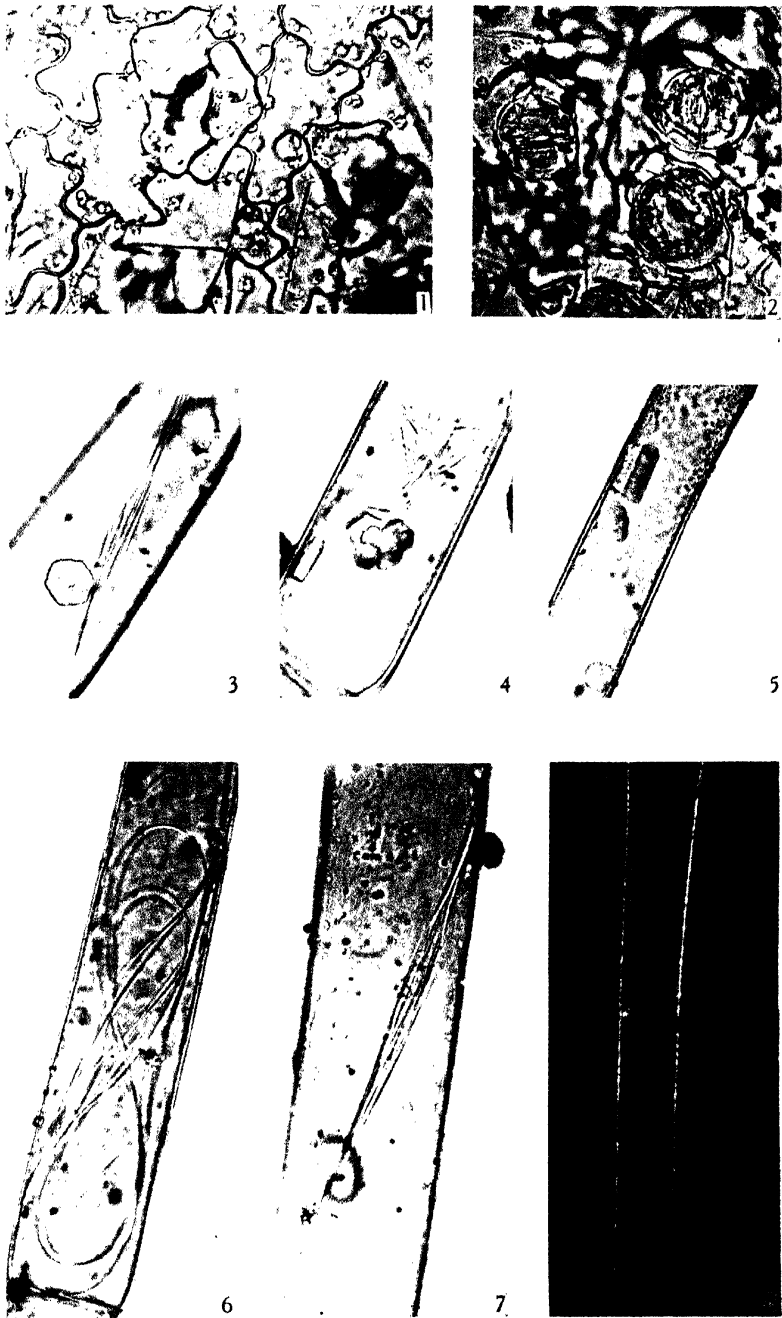
Until recently the only amorphous bodies which had been found in tobacco or enation mosaic infected plants had been of the amoeboid type. In December 1940 and the following weeks very large loosely packed masses of amorphous material were found (Pl. 14, figs. 3-5). This material was sometimes mixed with fibres. The fibres and some of the other particles were birefringent. The material in the masses was often closely packed but sometimes a hyaline vesicle with a few granules gave very much the appearance of an aucuba mosaic body immediately after pricking (Sheffield, 1939a). Also in the cell were often found large numbers of the small hyaline spherical bodies just described as occurring in aucuba mosaic. These usually contained highly refractive granules which were either spherical or rectangular. The contents of cells which contain these bodies are usually in such active movement that they are very difficult to photograph with an ordinary camera. The cytoplasm flowed rapidly carrying the smaller bodies with it. Particles within the large mass also moved. A small amount of striate material was sometimes found but more usually it was absent. In adjacent cells all the striate material was often found to have fused to a single mass. It is thought probable that these amorphous masses originate in striate material which has fused (as in Pl. 13, fig. 4) and undergone a change. Masses of this type were found in tobacco and tomato with all of the three strains of virus. They occurred most frequently in old tomato leaves especially in the cells of the large hairs of the petiole.

DISCUSSION

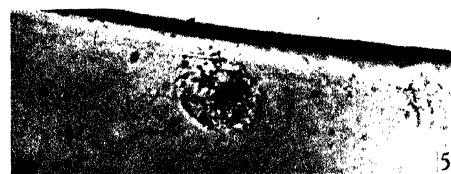
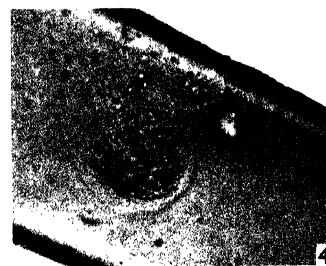
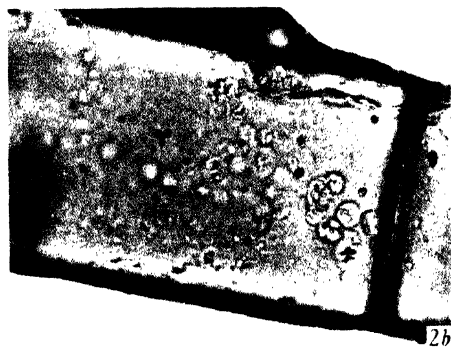
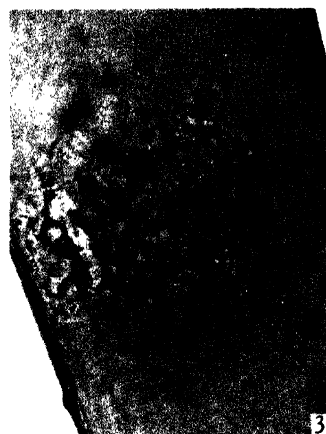
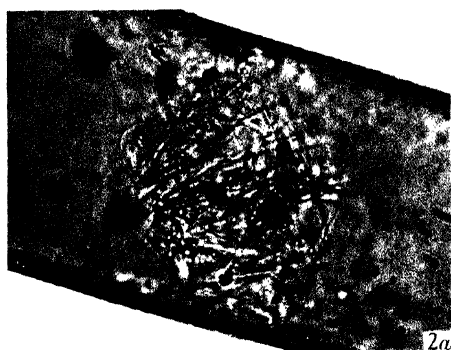
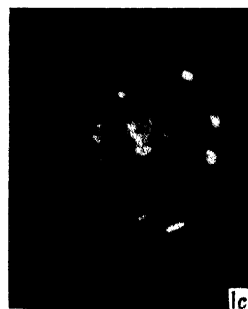
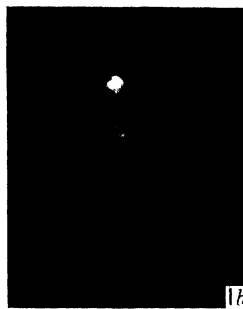
The variations recorded* cannot be due to mutation of the virus strain for almost identical results were obtained from all three strains. Also inoculations were made from material preserved at different times over a period of 13 years. All inoculations made at the same time gave similar results. Further, when an infected plant was kept for some months the reaction of the later formed leaves was often different from that of the first formed leaves.

Inclusions may be modified slightly in form according to the host containing them (Sheffield, 1931). Further evidence is now afforded of the influence of the host plant. In *S. nodiflorum* far less variation was shown than in other hosts: the greatest variety was shown by tomato, especially in the largest hair cells. It is possible that the conditions which control the form taken by the intracellular inclusions also to some extent control their

* Note added to proof. Every type of intracellular inclusion body described in this paper was observed again during the early summer of 1941.



KASSANIS AND SHEFFIELD—VARIATIONS IN THE CYTOPLASMIC INCLUSIONS INDUCED BY THREE STRAINS OF TOBACCO MOSAIC VIRUS (pp. 360-7)



KASSANIS AND SHEFFIELD—VARIATIONS IN THE CYTOPLASMIC INCLUSIONS INDUCED BY THREE STRAINS OF TOBACCO MOSAIC VIRUS (pp. 360-7)

distribution throughout the tissues of the host plant. Hirayama & Yuasa (1937), in Japan, reported the regular occurrence of inclusions in the guard cells of the stomata of plants infected with tobacco mosaic virus, but Sheffield (1936*a*) was unable to find them and thought that the differences might be due either to the virus strain used or to the climatic conditions. The latter appears to be the more probable cause, for inclusions have now been found in the stomatal guard cells of one such plant grown at Rothamsted. Although they have frequently been found here in solanaceous plants infected with severe etch virus (Kassanis, 1939; Sheffield, 1941) where large numbers of inclusions are found in almost every tissue of the plant, it is the first time that inclusions have been found at Rothamsted in the guard cells of any plant infected with a tobacco virus strain although they have often been looked for. Even in 1940 they occurred rarely and were found only in September in one tobacco plant which was inoculated in July with tobacco mosaic virus. They have not been found in plants infected with the aucuba or enation strains.

Variations in the inclusions can be correlated to some extent with growing conditions and also with external symptoms which are themselves modified by growing conditions. In *Nicotiana glutinosa*, infection with any of these strains has always been confined to necrotic lesions around the points of entry of the virus and no intracellular inclusions are produced (Sheffield, 1936*b*). In tobacco, infection with aucuba mosaic virus may be localized when no inclusions are formed, or it may be systemic when inclusion bodies are many and various. With any of the strains, when external symptoms are very definite as in summer, inclusions are large, numerous and most varied. However, plants may show almost no external symptoms but still contain inclusions, which are then smaller, fewer and mostly of one kind.

The plants inoculated during the last seven months and examined during the last four months of 1940 made it obvious that the form taken by the inclusions varies with the season, all new fibrous forms were produced during the summer and the modified amorphous forms occurred principally in winter in plants inoculated in early summer. As winter approached all plants tended to form striate material to the exclusion of other forms. It seems probable that the chief factors determining the form of the inclusions are light and temperature. These virus strains multiply most rapidly in quickly growing plants and in winter artificial light was often supplied. It was found some years ago that plants infected with aucuba mosaic virus sometimes formed striate material but never produced amorphous inclusions in winter unless given additional light. This was provided by means of $\frac{1}{2}$ or 1 kW. electric bulbs with suitable reflectors over a period of 4-8 hr. during the night: such light was used every winter from 1930 to 1938. Although it was insufficient to produce the rapid growth usual in summer, amorphous inclusions were completely formed in about three weeks after inoculation. During May, June, August and September of 1940 the total hours of bright sunshine recorded at Rothamsted were well above the average for even that time of year (Table 1). It was during these months that all the plants showing exceptional forms of inclusion body were inoculated: unfortunately no plants were kept in shade during these months.

Comment has been made on the disappearance of the spike-like body for a period of years. It was present during the summers of 1928-30 and the amount of sunshine recorded was well above the average for the first two and just average for the third of these years. From 1931 to 1934 no records were made of the occurrence of the spike, so that it is possible that it disappeared and reappeared. In 1931-2, the hours of bright sunshine were well below

the average, whilst in 1933 they far exceeded it. In 1935 the absence of the spike was recorded and it was not seen again until 1940 although it was frequently looked for during the intervening years. During these years sunshine was poor. If sunshine be the sole determining factor it is strange that so many forms of inclusion were found in 1940 and far fewer in 1929 when the actual hours of light were greater than in 1940. It seems probable that temperature is a second important factor. In 1940, whilst receiving more than the usual amount of light, plants were getting less heat than in previous years. During the summer of 1940, no artificial heating was supplied to the glasshouses, whilst in the winter of 1940-1 they were heated only at night sufficiently to prevent damage from frost. Prior

TABLE 1. *Hours of bright sunshine recorded at Rothamsted**

Figures in italics show deviations from the averages which were taken over 48 years

Year	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Totals
Av.	52.7	70.0	119.0	152.4	198.5	205.2	198.2	187.7	150.8	105.9	63.4	43.6	1547.4
1928	64.9 <i>+12.2</i>	100.2 <i>+30.2</i>	92.8 <i>-26.2</i>	127.3 <i>-25.1</i>	169.8 <i>-28.7</i>	230.0 <i>+24.8</i>	276.3 <i>+78.1</i>	193.0 <i>+5.3</i>	212.0 <i>+61.2</i>	126.5 <i>+20.6</i>	72.1 <i>+8.7</i>	48.9 <i>+5.3</i>	1713.8 <i>+166.4</i>
1929	39.5 <i>-13.2</i>	67.2 <i>-2.8</i>	184.7 <i>+65.7</i>	155.1 <i>+2.7</i>	261.0 <i>+62.5</i>	226.5 <i>+21.3</i>	243.7 <i>+45.5</i>	196.7 <i>+9.0</i>	206.0 <i>+55.2</i>	120.1 <i>+14.2</i>	78.0 <i>+14.6</i>	75.3 <i>+31.7</i>	1853.8 <i>+306.4</i>
1930	48.8 <i>-3.9</i>	59.1 <i>-10.9</i>	123.5 <i>+4.5</i>	114.8 <i>-37.6</i>	166.3 <i>-32.2</i>	242.6 <i>+37.4</i>	194.6 <i>-3.6</i>	226.0 <i>+38.3</i>	125.0 <i>-25.8</i>	134.9 <i>+29.0</i>	76.6 <i>+13.2</i>	31.2 <i>-12.4</i>	1543.4 <i>-4.0</i>
1931	64.8 <i>+12.1</i>	65.4 <i>-4.6</i>	153.6 <i>+34.6</i>	115.7 <i>-36.7</i>	172.6 <i>-25.9</i>	198.0 <i>-7.2</i>	157.8 <i>-40.4</i>	155.6 <i>-32.1</i>	120.6 <i>-30.2</i>	118.4 <i>+12.5</i>	68.9 <i>+5.5</i>	40.5 <i>-3.1</i>	1431.9 <i>-115.5</i>
1932	50.5 <i>-2.2</i>	67.6 <i>-2.4</i>	144.2 <i>+25.2</i>	131.3 <i>-21.1</i>	128.4 <i>-70.1</i>	215.5 <i>+10.3</i>	136.3 <i>-61.9</i>	191.5 <i>+3.8</i>	113.2 <i>-37.6</i>	104.1 <i>-1.8</i>	47.4 <i>-16.0</i>	56.2 <i>+12.6</i>	1386.2 <i>-161.2</i>
1933	70.4 <i>+17.7</i>	102.4 <i>+32.4</i>	196.9 <i>+77.9</i>	153.4 <i>+1.0</i>	168.2 <i>-30.3</i>	240.6 <i>+35.4</i>	246.2 <i>+48.0</i>	243.2 <i>+55.5</i>	183.3 <i>+32.5</i>	94.6 <i>-11.3</i>	51.3 <i>-12.1</i>	41.4 <i>-2.2</i>	1791.9 <i>+244.5</i>
1934	56.9 <i>+4.2</i>	96.1 <i>+26.1</i>	127.0 <i>+8.0</i>	120.8 <i>-31.6</i>	200.8 <i>+2.3</i>	184.9 <i>-20.3</i>	274.8 <i>+76.6</i>	180.4 <i>-7.3</i>	172.6 <i>+21.8</i>	85.0 <i>-20.9</i>	45.9 <i>-17.5</i>	20.9 <i>-22.7</i>	1566.1 <i>+18.7</i>
1935	46.7 <i>-6.0</i>	53.0 <i>-17.0</i>	134.3 <i>+15.3</i>	126.7 <i>-25.7</i>	193.8 <i>-4.7</i>	195.0 <i>-10.2</i>	280.1 <i>+81.9</i>	203.9 <i>+16.2</i>	149.9 <i>-0.9</i>	112.1 <i>+6.2</i>	61.9 <i>-1.5</i>	47.5 <i>+3.9</i>	1604.9 <i>+57.5</i>
1936	49.6 <i>-3.1</i>	81.0 <i>+11.0</i>	86.0 <i>+33.0</i>	126.8 <i>-25.6</i>	177.0 <i>-21.5</i>	182.8 <i>-22.4</i>	120.9 <i>-77.3</i>	181.2 <i>-6.5</i>	84.4 <i>-66.4</i>	97.1 <i>-8.8</i>	46.3 <i>-17.1</i>	59.7 <i>+16.1</i>	1292.8 <i>-254.6</i>
1937	44.4 <i>-8.3</i>	64.4 <i>-5.6</i>	104.5 <i>-14.5</i>	95.3 <i>-57.1</i>	158.3 <i>-40.2</i>	187.6 <i>-17.6</i>	126.1 <i>+27.9</i>	187.1 <i>-0.6</i>	138.7 <i>-12.1</i>	78.3 <i>+27.6</i>	69.3 <i>+5.9</i>	24.1 <i>-19.5</i>	1278.1 <i>-269.3</i>
1938	47.1 <i>-5.6</i>	67.0 <i>-3.0</i>	176.6 <i>+57.6</i>	157.1 <i>+4.7</i>	161.4 <i>-37.1</i>	203.1 <i>-2.1</i>	143.4 <i>-54.8</i>	151.1 <i>-36.6</i>	120.0 <i>-30.8</i>	117.7 <i>+11.8</i>	68.9 <i>+5.5</i>	45.4 <i>+1.8</i>	1458.8 <i>-88.6</i>
1939	45.7 <i>-7.0</i>	106.0 <i>+36.0</i>	95.6 <i>+23.4</i>	164.8 <i>+12.4</i>	159.1 <i>-39.4</i>	205.2 <i>-40.7</i>	157.5 <i>-36.0</i>	151.7 <i>-8.8</i>	142.0 <i>-15.9</i>	90.0 <i>-25.4</i>	38.0 <i>+0.6</i>	44.2 <i>-147.6</i>	1399.8 <i>-147.6</i>
1940	86.7 <i>+34.0</i>	22.5 <i>-47.5</i>	127.1 <i>+8.1</i>	124.0 <i>-28.4</i>	224.8 <i>+26.3</i>	267.9 <i>+62.7</i>	180.8 <i>-8.4</i>	191.1 <i>+3.4</i>	170.9 <i>+20.1</i>	93.9 <i>-12.0</i>	76.6 <i>+13.2</i>	41.2 <i>-2.4</i>	1616.5 <i>+69.1</i>

* This table was adapted from data published in the *Annual Reports of the Rothamsted Experimental Station*.

to 1940, heat was provided all the time during the cooler months and at the time these observations commenced, it was turned off only during periods of exceptional heat during the summers. It is clear that a certain minimum of both heat and light is necessary for the formation of inclusions, and it is possible that the form of these might be modified by changing the balance between the amounts of heat and light made available to the plants. It is not suggested that variation could not be brought about in other ways such as by the supply of nutrients available. This is however unlikely to be the cause of the variations recorded here as all the experimental plants described were grown in rather rich soil.

If the type of inclusion body is controlled by environmental conditions, it would be expected that those new types now described would have been found previously by workers

in countries which have a greater amount of sunshine than is experienced here. Except the 'raphides' no fibrous forms have ever been mentioned. The amorphous bodies noted by Hirayama & Yuasa (1937) may be similar to those described in this paper. It is possible that other types have occurred but have been destroyed or distorted by the technique employed in examining infected cells. Most of the cytological observations on tobacco mosaic disease have been made on fixed material and the fibrous forms would be destroyed by any but the least acid fixatives. The new amorphous forms (Pl. 14, figs. 2-6) would be extremely difficult to fix in the large vacuolated cells which usually contain them and would in all probability be misinterpreted.

It has never been possible to find any differences between the hexagonal crystals usually produced by the three strains. These crystals and the more usual type of amorphous inclusion body induced by aucuba mosaic virus obviously contain some constituent in common. A variety of previously unrecorded forms has now occurred. These types are similar with all three virus strains. Moreover, they are often derived from pre-existing bodies of the better known types. It appears that, although they differ in morphology, there can be little essential difference between any of the inclusions formed by these three virus strains.

SUMMARY

According to previous accounts tobacco mosaic virus regularly induced striate material and amoeba-like inclusions and occasionally raphides in the host cells; enation mosaic virus gave striate material and amoeba-like *X*-bodies; whilst aucuba mosaic virus induced either striate material or a large amorphous inclusion which later gave rise to striate material. A spike-like body recorded in the early descriptions of aucuba mosaic disease had not been seen for some years. In 1940, a variety of new forms were induced by all three strains. These new forms were mostly fibrous. The spike-like body reappeared, spindle-shaped bodies, masses of short needle-like fibres and extremely long coiled fibrous forms occurred. New amorphous forms were also found. All these arose either directly or from pre-existing inclusions of the previously recorded types. Variation in the inclusions produced is not due to mutation of the virus. The type of inclusion is to some slight extent determined by the host plant but seems to be largely controlled by the amount of light and heat available to the host.

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EXPLANATION OF PLATES 13 AND 14

All preparations were made from unstained living material. Pl. 13, figs. 1 and 2, are epidermal strippings: all other figures show parts of hair cells from petiole or lamina. All photographs were taken with a Leitz Mikam camera. A Leitz 6L objective (N.A. 0.65) was used in conjunction with a Leitz 10× periplanatic ocular giving a magnification of 450× on the negative. Contact prints are reproduced without alteration in size. The source of illumination is given in brackets after the description of each figure: 'MV' signifies a mercury vapour lamp, and 'Monla', a Leitz lamp of that name; the numbers refer to Wratten filters.

PLATE 13

- Fig. 1. Tobacco infected with tobacco mosaic virus. Long spike-like bodies, plastids and a few crystalline inclusions are seen. (MV, 62.)
- Fig. 2. Tobacco infected with tobacco mosaic virus. Spike-like bodies and small crystalline inclusions are present in the guard cells of the stomata. (MV, 62.)
- Fig. 3. Tobacco infected with tobacco mosaic virus. A crystalline inclusion and a mass of long fibres occupy the centre of the cell. (MV, 62.)
- Fig. 4. Tobacco infected with tobacco mosaic virus. A mass of needle-like fibres is present; lying against the cell wall is a hexagonal crystal which in edge view appears rectangular; in the centre is a shapeless mass formed by the aggregation of hexagonal crystals. (MV, 62.)
- Fig. 5. Tomato, 8 months after infection with enation mosaic virus. Hexagonal crystals in edge view show distinct striations although untreated with acid. (MV, 62.)
- Fig. 6. Tomato infected with aucuba mosaic virus. Long fibres curve to form a figure 8. (Monla, 58 and 22.)
- Fig. 7. Tomato infected with aucuba mosaic virus. A spindle-shaped body appears to be an aggregation of long spike-like fibres. (Monla, 58 and 22.)
- Fig. 8. Tomato infected with aucuba mosaic virus. A long doubly refractive fibre lies parallel to the cell wall and close to it. (Monla, between crossed Nicol prisms.)

PLATE 14

- Fig. 1*a*. Tomato infected with aucuba mosaic virus. An inclusion body (approx. 70 μ diam.) contains amorphous material and a few small crystals. (Monla, 58 and 22.)
- Fig. 1*b*. Inclusion in fig. 1*a*, seen between crossed Nicol prisms; the crystals and a little of the granular material are birefringent. (Monla, between crossed Nicol prisms.)
- Fig. 1*c*. As fig. 1*b*, 30 hr. later. More birefringent material is apparent.
- Fig. 2*a*. Tomato infected with aucuba mosaic virus. A large amorphous body gives rise to fine needle-like fibres and also to hyaline spherical bodies, each containing a few highly refractive granules. (Monla, 58 and 22.)
- Fig. 2*b*. Another part of the cell shown in fig. 2*a*. Large numbers of small hyaline spheres each containing a few granules float in the cell sap. (Monla, 58 and 22.)
- Fig. 3. Tomato, 8 months after infection with enation mosaic virus. A large mass seems to consist of amorphous material and small hyaline spheres, some of which contain minute round or rectangular particles: from the mass project hyaline bodies. (MV, 62.)
- Fig. 4. Tomato, 7 months after infection with tobacco mosaic virus. A body is partly hyaline and partly contains granular material. (MV, 62.)
- Fig. 5. Tomato, 8 months after infection with enation mosaic virus. An inclusion is very similar to that shown in fig. 4: hyaline bodies also are present. (MV, 62.)
- Fig. 6. Tomato, infected with aucuba mosaic virus. The cell contains an amoeboid body similar in appearance to the X-bodies of tobacco and enation mosaics: part of a long fibre also seen. (Monla, 58 and 22.)

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II.—THE CYTOPLASMIC AND NUCLEAR INCLUSIONS ASSOCIATED WITH SEVERE ETCH VIRUS.

576.807.71

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THREE PLATES.

INTRODUCTION.

ALTHOUGH over 100 plant virus diseases have been described, in less than one-fifth of these have abnormal inclusions been described. No doubt they occur in some others, but in many they have not been found, although an extensive search has been made. This is in striking contrast to virus diseases of animals, in almost all of which inclusions have been found. In the animal diseases inclusions in the nuclei are as common as those in the cytoplasm. In plant diseases, on the other hand, there had been no convincing demonstration of intranuclear inclusions until recently, when Kassanis (1939) found intranuclear crystals to be a constant symptom of infection with severe etch virus. Kassanis compared these in size, stability, and staining reactions with the intranuclear inclusions of the polyhedral disease of silkworms. He also found many cytoplasmic inclusions of the same type as the amorphous bodies produced by some strains of tobacco mosaic and some other plant viruses. This paper describes some further properties of the two kinds of inclusions induced by severe etch virus.

MATERIAL AND METHODS.

Most of the work was done with *Nicotiana tabacum* var. White Burley, but *N. glutinosa* and *Hyoscyamus niger* were also used.

Living tissues were usually examined by transmitted light, but occasionally dark ground illumination or polarized light was used.

Micrurgical methods, similar to those employed in the examination of some of the inclusions produced by strains of tobacco mosaic virus (Sheffield, 1939), were used.

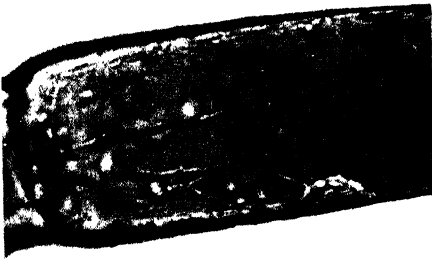
Microtome sections of many different tissues were examined. The fixative used for most of these was Champy's fluid, which was known to give good results with these hosts (Sheffield, 1938). Kassanis (1939) stated that the plate-like crystals were destroyed by acetic acid, so fixatives containing this were at first avoided. It was later found to be true of only very high con-

centrations of acid, and then Bouin's fixative and also Allen's modification were used with good results, but the chondriome was, of course, not preserved. Formol-saline as suggested by Kassanis gave only poor results when material was fixed in bulk. It was found to be a little more successful but not very good for epidermal strippings. As Kassanis found, Kull's staining method gave very spectacular results, and this was used for many of the preparations. In attempts to differentiate between the various normal and abnormal cell constituents many staining combinations were used. These are detailed in Table III (p. 39).

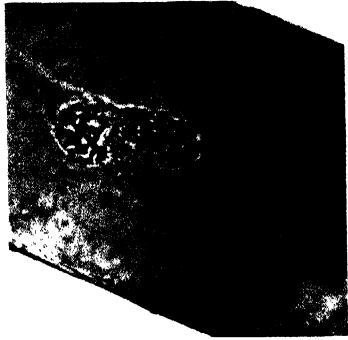
Attention might be drawn to one of them. Difficulty is often experienced in differentiating between the nucleus and cytoplasmic inclusion bodies. Feulgen's reagent with a counterstain is often used for this purpose, as the nucleus invariably stains with leuco-basic fuchsin whereas all the virus inclusions in plants yet tested fail to do so. Carbol fuchsin (basic fuchsin in phenol) differentiated in picric acid in clove oil provides a similar but further differential stain for some bodies. It was first used for plants infected with severe etch virus when the nucleus stained red and the amorphous inclusions yellow. Similar results were obtained with hosts infected with aucuba and tobacco mosaic and with Hy. III virus. Actually it is not as good for severe etch material as some others, for the red of the chromatin tends to be rather diffuse and to obscure the pale yellow of the intranuclear inclusions.

DESCRIPTION.

Morphology.—The amorphous bodies look like those induced by aucuba mosaic or Hyoscyamus III viruses. When first formed they are diffuse but later become more compact. Generally they are larger than the inclusions of aucuba mosaic and are more often ovoid than spherical (Pl. I, figs. 2-3). This is probably often a sequel to their large size, the width of the cell being insufficient to contain them if they were spherical. Soon after infection small granules appear in the cytoplasm, and the bodies are built up by these coalescing in the same way as the amorphous bodies produced by aucuba mosaic or Hy. III viruses. Usually they do not become vacuolate; when they do it is at a late stage preparatory to dissolution (Pl. I, fig. 6). They are less homogeneous than the aucuba mosaic bodies and obviously consist of large numbers of minute particles, many of which are doubly refractive when viewed between crossed Nicol prisms. The bodies are more stable than those of aucuba mosaic, which are immediately destroyed by pressure on the cell wall in the region of the body (Sheffield, 1939). Such treatment causes the dispersal of the particles composing the severe etch body but not their dissolution (Pl. III, fig. 5). The aucuba mosaic inclusion is immediately destroyed by pricking. Within a few seconds it either disappears or else a hyaline vesicle is formed which disappears in the course of a few minutes. By contrast a microneedle can be thrust into or through a severe etch inclusion with no apparent effect (Pl. III, fig. 3). By micromanipula-



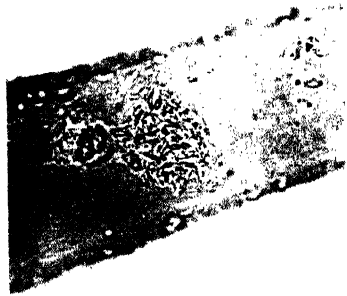
1



2



3



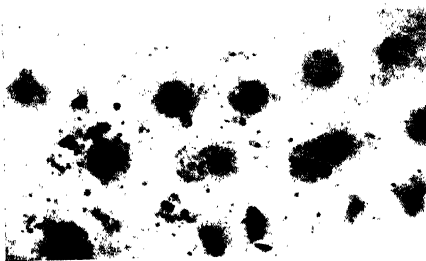
4



6



5



8



7

[To face p. 32.]



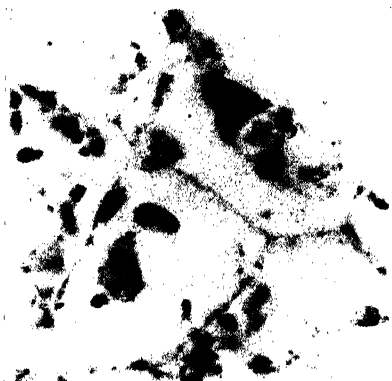
1



2



3



4



5



6a



6b

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tion such a body can be divided into portions, each of which will then persist unchanged (Pl. III, fig. 4).

The intranuclear inclusions take the form of rectangular (often square) plates (Pl. I, figs. 3 and 7 and Pl. II, fig. 4). When first formed their sides may be as little as 1μ in length (Pl. I, fig. 7). They increase in size, and their increase would seem to be analogous to the growth of crystals in a saturated solution. Some grow until they can only just be accommodated within the nucleus, their sides then measuring about 10μ . They are extremely thin, the largest being less than 0.5μ in thickness. Because they are thin a large number can be packed within a single nucleus. The nucleus of a very young cell may contain only a single small crystal (Pl. I, fig. 7), but in adult cells several usually occur together (Pl. I, figs. 2-5). Kassanis (1939) has described 15 within one nucleus, and it is difficult to count a greater number than this by direct observation of the nucleus within the cell. In nuclei that were withdrawn from adult cells by a micro-needle and then either torn to pieces or immersed in water until the nucleus burst open, however, more than 30 crystals were often found in one nucleus. The crystals withstand much maltreatment. They can with difficulty be crushed by pressure from the tip of a microneedle, when they leave a shapeless mass.

Mode of Formation.—The time of the first appearance of inclusions varies with the age of the plant and with the conditions of growth. Under poor light conditions and at low temperatures the production of inclusions, as of many symptoms, may be inhibited or considerably delayed. The intranuclear inclusions always appear before the cytoplasmic ones. In the summer rubbed leaves usually show yellow lesions within five days, and on the sixth day the veins of younger leaves become cleared. At this time no abnormalities can be seen, either in the nuclei or in the cytoplasm. On the following day as many as a dozen crystals may be counted in many of the nuclei. At the same time, the cytoplasm is seen to be very conspicuous and its streaming greatly accelerated, and minute, highly refractive particles can be seen in it (Pl. I, fig. 1). These particles are carried in the cytoplasmic stream, and when they meet they fuse and large masses are quickly built up. The process is often much more rapid than with aucuba mosaic virus. Single large masses may be produced within two days of the appearance of external symptoms. These become slightly more compact and usually assume an ovoid form, although the surface often remains irregular. Cells of *Hyoscyamus niger* infected with severe etch virus behave similarly to those infected with aucuba mosaic virus, the inclusions often remaining as a number of diffuse masses spread throughout the cell.

Kassanis (1939) had found both cytoplasmic and intranuclear inclusions to be absent from the growing points. It seemed possible that, if the development of the cell were followed from the meristematic stage until it reached maturity, some information as to the origin of the intranuclear inclusions might be derived.

In the leaf of the healthy tobacco plant the cells remain meristematic

only until the leaf is about 3 mm. in length. After this no nuclear or cell division occurs and growth is due to increase in size and separation of the cells. When meristematic, the cells are small and closely packed. Each contains a nucleus which occupies a large portion of the centre of the cell. It is surrounded by viscous cytoplasm with numerous tiny vacuoles. By appropriate methods chondriosomes and proplastids can be demonstrated in the cytoplasm. When mitosis and cytokinesis cease, the cells enlarge and assume the form appropriate to their function. The nuclei do not enlarge, nor does the amount of cytoplasm appear to increase. The vacuoles increase in size and join together until thin layers of cytoplasm line the walls and surround the nucleus and a few strands cross the vacuoles. At the same time in certain cells the plastid primordia develop gradually into plastids.

In plants infected with severe etch virus the meristematic growth of the young stem and leaves appears to be quite normal. After the telophase of the last division, when the nuclear membrane is reformed and the chromosomes commence to lose their identity, several small nucleoli appear in close contact with certain of the chromosomes. As the nucleus passes into the resting stage these nucleoli fuse together to form usually a single large body, but occasionally two, or, more rarely, three. It is when the nucleoli are appearing in the reconstituted nucleus following the last meristematic division that the first cytological abnormalities are observed in infected plants. At the same time small plate-like bodies appear in the nuclei and rapidly increase in size and number. They appear to crystallize from the cell sap and are not formed in contact with chromosomes, as are nucleoli.

In many of their reactions the crystals behave similarly to the nucleoli. It therefore seemed possible, especially as the former appear almost immediately after the latter, that the intranuclear inclusions were formed at the expense of the nucleoli. But the production of crystals does not result in any diminution in the number or size of the nucleoli, and in adult cells containing many large crystals the nuclear structure seems to be otherwise exactly as in the cell of the healthy plant. It then seemed possible that infection resulted in an excessive production of nucleolar material, but their staining reactions (Table III) show that although the two substances are similar they are not identical.

Besides inducing crystal formation, infection may also induce further mitoses. These usually follow rapidly on what would normally be the final cell division but sometimes occur in cells already differentiated. When, after the prophase, the nuclear membrane disappears the crystals are then thrown out into the cytoplasm. Apart from lagging of some of the chromosomes at anaphase the division appears to be quite normal until the telophase. Then the daughter nuclei are reconstituted in the usual way but no cell plate is formed across the centre of the spindle and cell division does not occur. A similar karyokinesis which is not followed by cytokinesis occurs in *Nicotiana glutinosa*, when necrotic lesions are formed as a result of rubbing the leaf surface with suspensions of strains of tobacco mosaic virus (Sheffield,

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1936). Such divisions result in the production of binucleate cells. As soon as the daughter nuclei are reconstituted, further crystals appear in them. The crystals released from the parent nucleus remain for a time in the cytoplasm. It is not known whether they later dissolve, but such bodies were found only occasionally in adult cells. If all persist it would be expected that they would be observed more frequently.

In some respects these nuclear inclusions seem comparable to the protein crystals which occur regularly in the nuclei of certain healthy plants. In the case of Rivina, Stock (1892, *cf.* Tischler, 1934) was able to induce the production of similar but extranuclear crystals by giving excessive nitrogen. Tobacco plants were therefore given a weekly top dressing of 0.6 gm. NaNO_3 . In some the dressings began before inoculation, in others at the time of, and in others, after infection. In no case was the formation of extranuclear inclusions induced. In some cases the treatment caused the cytoplasm to become increasingly conspicuous.

Distribution.—Inclusion bodies are often confined to certain tissues. Those of aucuba mosaic are most prevalent in the tegumentary tissues (Sheffield, 1931). They occasionally occur in the palisade parenchyma but rarely in other tissues. They are not found in the meristem. Those of tobacco mosaic are more widespread, and occur even in actively growing tissue (Goldstein, 1926), as also do those of mosaic-infected dahlia plants (Goldstein, 1927). Those induced by Hy. III virus which bear many similarities to severe etch are relatively numerous and occur in many tissues. This similarity extends to the amorphous inclusions caused by severe etch virus which are numerous in all tissues. Often the cells containing them do not attain their full size. As they are usually contained by almost every cell over large areas of tissue, an uneven growth follows, resulting in deformity of individual leaves and stunting of the plant.

The intranuclear inclusions of severe etch virus are even more widespread than any other virus inclusions so far recorded. The intranuclear inclusions in animals infected with viruses are usually confined to certain organs but those of severe etch occur in almost every tissue and are contained by most of the cells. Both types of inclusion are rare in very old tissues but are abundant in all those tissues which were in an actively growing condition at the time of inoculation or which were produced after infection. Neither type occurs in the tissue which is normally meristematic, although intranuclear inclusions are found in cells where mitosis is abnormally induced. Both types occur in tegumentary tissues, including the hairs, epidermal cells, and guard cells of the stomata, and in the assimilating tissue and general ground tissues of the leaf, stem, and root. In the vascular bundles they have not been found in the xylem vessels but are prevalent in xylem parenchyma and phloem. In the sepals and petals both types occur as frequently as in the leaves. In all these organs intranuclear inclusions are found in almost every cell and amorphous inclusions in many of them.

Because the inclusions of severe etch were found to be so widespread a

special examination was made of the reproductive organs. Inclusions of both types occur frequently in the filaments and more rarely in the anther walls, but none was observed in the tapetum. Neither do they appear to be formed in the pollen-mother cells, nor in the pollen grains either before or after nuclear division. They are abundant in the ovary wall, also in the style, but although they occur just below the stigma they were not found in the cells composing it. They could not be found in young ovules, either in the nucellus or embryo-sac. In the seed they were not found in the endosperm or in the young embryo. Nuclear, but not cytoplasmic, inclusions were of frequent occurrence in the seed coat.

Although inclusions were not found in other parts of the seed, their presence in the testa suggested a possibility of the presence of virus in other parts of the seed. Tests for seed transmission of the virus were therefore made. Seeds from infected tobaccos were sown immediately on ripening. None of the 408 seedlings obtained showed any virus symptoms, and inoculations made from them to healthy tobaccos all gave negative results. 198 seedlings obtained from seed of infected *Hyoscyamus niger* were also healthy.

Seed taken at the time of ripening, crushed, diluted to 1 in 10, and inoculated to healthy tobaccos gave no infections. The virus did not appear to be inactivated by other seed contents as crude infective juice diluted with seed extract gave as many infections as juice diluted with water. As the virus is rapidly destroyed by desiccation it seemed more probable that it was inactivated by the drying out which occurs in the natural course of ripening of the seed. This was confirmed when unripe seeds taken from infected tobaccos, crushed, and inoculated to healthy plants gave infections.

Persistence.—Most writers on inclusion bodies have been concerned mainly with their morphology and chemical reactions. Little has been written about their formation and persistence. It is known that the inclusions of some viruses disappear after an interval. Neither the amoeboid bodies of tobacco mosaic nor the striate material are found in very old plants. The amorphous inclusions of aucuba mosaic crystallize after a few days or weeks, and later the crystals dissolve. The inclusions of Hy. III virus disappear sometimes, giving rise to needle-like bodies.

With severe etch virus both types of inclusion body disappear from the oldest leaves, but they continue to be produced in the young tissues of tobacco plants even eighteen months after infection.

Needle-like bodies may be formed within the cytoplasmic inclusions in plants infected with severe etch virus. In the majority of leaves they are of rare occurrence but in certain types occur regularly. Severe etch causes the deformation of some leaves, the laminae being misshapen or reduced to extremely narrow strips along each side of the midribs. In other leaves which are of normal shape the sticky secretion normal to the tobacco plant is greatly reduced, so that the leaves appear almost glabrous. In these types the cytoplasmic inclusions almost invariably contain the needle-like bodies.



1a



2a



3



1b



2b



4



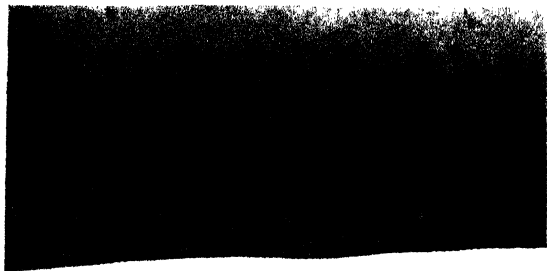
1c



2c



1d



5

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The needles may be from 1–5 μ in length and are extremely narrow (Pl. I, figs. 4–6). When viewed between crossed Nicol prisms they are seen to be doubly refractive. In older leaves inclusions containing these needles also contain numerous smaller birefringent particles which show active Brownian movement within the amorphous inclusions, suggesting a more liquid phase containing numerous particles, many of which are needle-like. Later these bodies become vacuolate, and the needles are then seen in the reticulum so formed (Pl. I, fig. 6). This is thought to be a stage in the complete dissolution of the amorphous inclusion body.

Virus Content of the Amorphous Inclusions.—Cells were mounted in 0.1M phosphate buffer at pH 7 and their amorphous inclusions extracted from them.* The inclusions were then suspended in water, in which they broke up, and stored until a sufficient number were collected. So far as was possible, roughly spherical inclusions of about 20 μ diameter were chosen. When 20 were collected the suspension was diluted to 1.6 c.c. Assuming their specific gravity to be about unity, this gave a concentration of approximately 1 gm. in 2×10^7 c.c. 0.8 c.c. of this was further diluted to 1.5 c.c. to give a dilution of 1 in 10^8 . These dilutions were then rubbed over the leaves of young tobacco plants and compared for infectivity with a $\frac{1}{200}$, and a $\frac{1}{1000}$ dilution of crude infective sap. A water control was also included. After an appropriate interval (determined afresh for each experiment by inoculating plants on the day prior to the main experiment and making daily tests on the leaves) leaves were tested for starch lesions. This experiment was repeated three times. The results are summarized in Table I.

TABLE I.
INFECTIVITY OF AMORPHOUS INCLUSIONS OF SEVERE ETCH VIRUS.

Inoculum.								Total numbers of starch lesions.
Water	0
Inclusions at dilution of 1 in 10^8	27
" " " 1 in $2 \cdot 10^7$	91
Crude juice at dilution of 1 in 10^8	29
" " " 1 in $2 \cdot 10^2$	147

Comparisons were also made of the infectivity of the inclusion body and of the remainder of the cell. Halves of cells containing inclusions were isolated. Also, those halves of inclusion-containing cells which were devoid of inclusions were collected. 20 half-cells of each series were suspended in 1.6 c.c. Comparisons were made with a water control and also with a 1 in $2 \cdot 10^2$ dilution of crude infected sap. The results of two experiments are shown in Table II.

* Method as described by Sheffield, 1939.

Transactions of the Society.

TABLE II.

Inoculum.	Total numbers of starch lesions.
Water	2
Half-cells with inclusions	19
Half-cells devoid of inclusions	12
Crude juice at dilution of 1 in 2·12 ³	72

No tests were made on the infectivity of the intranuclear inclusions. It was not difficult to isolate them, either separately or in groups as contained within the nuclei, but no suitable method of dissolving them is known. The known solvents would inactivate any virus which might be present. They can be broken mechanically but only if handled individually. They are too small for there to be any certainty of breaking them if handled in mass. Until a suitable method of suspension is found it would be unwise to attempt to test their infectivity.

Chemical Properties and Staining Reactions.—Like the amorphous inclusions of aucuba mosaic (Sheffield, 1933), those of severe etch virus obviously contain mixtures of different substances. By various techniques of fixing and staining the presence of chondriosomes and oil globules can be demonstrated, the latter appearing to be rather more abundant in the severe etch inclusions than in those of aucuba mosaic. Unlike the bodies of aucuba mosaic, those of severe etch contain particles which are doubly refractive when seen in polarized light. The intranuclear inclusions are in the main homogeneous. By a technique such as Kolachev's osmic impregnation method occasional small dark staining particles may be found within them (Pl. II, fig. 4). Careful examination between crossed Nicol prisms shows these plate-like bodies to be doubly refractive when seen in edge view but not when viewed flat (Pl. II, figs. 6a and 6b).

In strong acids such as hydrochloric, the intranuclear inclusions are soluble, and also in strong alkalis such as 10 p.c. caustic potash. The amorphous bodies are unaffected by strong acids but part of their substance is soluble in alkali. More delicate tests on the effects of slight amounts of acids and alkalis were made by removing the inclusions from the cells and suspending them in solutions of known pH. In the case of the amorphous inclusions these had to be of an osmotic pressure not less than that of the cell sap, for lower pressures cause dissolution of the bodies. The intranuclear inclusions persist unchanged for many hours after suspension in distilled water. They are unaffected over a pH range from 10 to 2. The amorphous inclusions of both aucuba mosaic and severe etch viruses are unaffected by increasing acidity (Pl. III, figs. 2 a-c), but at pH 10 a large portion of the body is soluble. Bodies suspended in such solutions rapidly shrink to a fraction of their original size. At pH 8 the shrinkage is more gradual. Some portion of the aucuba mosaic inclusions appears to be soluble in weak alkalis. If a body is removed into a phosphate buffer solution at pH 8 and 0·1 molarity, it

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appears first to swell, a portion of the contents seems to liquefy. Then shrinkage occurs, the amount of solid matter remaining unchanged but the liquid seeming to be withdrawn (Pl. III, figs. 1a-d). The shrinkage of these bodies seems to be less than that of the severe etch inclusions.

Neither type of severe etch inclusion is affected by alcohol and both are preserved by Carnoy's fluid, which contains absolute alcohol, chloroform and glacial acetic acid in the ratio of 2 : 3 : 1.

By ether the intranuclear inclusions are unaffected but part of the amorphous inclusion is soluble. The reactions with Sudan III and Scharlach R and osmic acid suggest the presence of fats in the cytoplasmic but not in the nuclear inclusion. Both types give positive reactions with Millon's reagent and with the xanthoproteic test, suggesting the presence of large quantities of protein in each type of inclusion body. As with other plant virus bodies Feulgen's reagent drew a sharp contrast between the protein of the inclusion bodies and that of the nucleo-proteins normally occurring in the nucleus. Neither type of inclusion restored the colour to leuco-basic fuchsin but the chromatin assumed a purplish-red colour.

As the nucleolus and the intranuclear inclusions behave similarly with many reagents it seemed possible that some relationship exists between them. The behaviour of the small nucleoli in young nuclei in which crystals are forming and the presence of large nucleoli in nuclei containing up to thirty inclusions, precludes the possibility of the inclusions being formed at the expense of the nucleoli. The possibility that infection induces an excessive production of nucleolar material which takes a crystal form was tested by the trial of many staining reactions. The results of these tests are summarized in Table III.

The reactions of the amorphous inclusions vary according to the method of fixation employed. A matrix of similar but deeper staining properties to the cytoplasm was often discerned but the presence of contrasting particles within depended on the technique employed. Stains for the chondriome were used only after appropriate fixing methods, although staining techniques which destroy chondriosomes were employed after both the less and the more acid fixatives. With this explanation it was not thought necessary to tabulate also the fixatives. The intranuclear inclusions were preserved by and behaved similarly after all those employed.

It should also be remembered that any particular structure may vary in its staining capacity according to the method and degree of differentiation required by the staining combination in use. With acid fuchsin, for example, the nucleolus stains red, but washing with water removes the stain, the nucleolus becoming colourless whilst the crystals are still red. With Kull's combination the result is similar. When the same dye is used with crystal violet, differentiation is in picric acid and the red colour is allowed to remain in the nucleoli; but when in combination with methyl green, the acid fuchsin is removed and the crystals and nucleolus allowed to take the green stain.

TABLE III.—STAINING REACTIONS.

Dyes.		Reactions.				
Basic.	Acid.	Name or author of technique.	Chromatin.	Nucleolus.	Nuclear Inclusion.	Cytoplasm.
Leuco-basic fuchsin (after hydrolysis) *	—	Feulgen	Red	Colourless	Colourless	Colourless
Ditto	Orange G.	Feulgen with counterstain	Red	Orange	Orange	Orange
Ditto	Picric acid	Ditto	Red	Yellow	Yellow	Yellow
Ditto	Light green	Ditto	Red	Yellow Green	Green	Green
Ditto	Light green (mordant and differentiator in Na_2CO_3 solution).†	Semmens and Bhaduri (1939)	Red	Green	Green	Colourless
Basic fuchsin in phenol (after 1 p.c. HCl)	Picric acid in clove oil (used to differentiate basic stain)	Zieth modified by Lenoir (1932)	Pink reticulum Bluish-red chromosomes	Red	Yellow	Colourless-yellow
Methyl green	Acid fuchsin	Guignard (cf. Bolles Lee)	Reticulum pink green Chromosomes	Colourless-red Green	Red Green (pale)	Colourless Pink (pale)
Crystal violet	Ditto (differentiate in picric acid)	Nebel (cf. Bolles Lee)	Purple	Red	Yellow-red	Red
—	Methyl-blue } Mixed Eosin	Mann	Blue red overlays	Red	Red	Red
38 Methyl green (mixed) Pyronin B (differentiated in resorcin)	—	Pappenheim	Green overlays red	Green overlays red	Green	Colourless-pale green
Pyronin B (differentiate in resorcin)	—	—	Red	Red	Colourless	Colourless
Magdala red	Light green	—	Red	Colourless-green Purple	Colourless-green Purple	Pale green Orange
Thionin in phenol	Orange G in absolute alcohol (used to differentiate basic stain)	Stoughton	Blue	Red	Red (more intense than nucleolus)	Red granules and globules in matrix of yellow-green
Toluidine blue	Acid fuchsin (in aniline oil water)	Kull	Blue	Colourless-black	Colourless-black	Chondriosomes and other granules and globules black in colourless matrix
Hematoxylin (mordant and differentiate in iron alum)	Aurantia	Heidenhain	Black	Colourless-purple	Colourless-purple	Purple granules
Gentian violet (mordant and differentiate in iodine in alcoholic KI)	—	Newton	Purple	Colourless-purple	Colourless-purple	Colourless matrix containing very many black particles
Gentian violet (mordant 2 p.c. eq. tannin)	Osmic acid (impregnation method, 7 days at 40° C.)	Chen	Purple	Colourless-purple	Colourless-purple	Colourless matrix containing very many black particles
—	—	Kolachev	Black	Brown	Brown	Colourless with black chondriosomes, etc.

* As the usual method of hydrolysis for 4 min. at 60° C. caused shrinkage in the nuclei, this method was modified, hydrolysis being carried out at room temperature over a longer period (about 1 hour).

† The results given by this method were better defined than when the light green was used as a simple counterstain.

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The nucleolus and the nuclear inclusions reacted similarly to many of the stains used. However, with Pappenheim's stain the nucleolus seemed to be red and overlaid with green, whilst the inclusion appeared to have taken the green dye only. Preparations single-stained with pyronin B showed red nucleoli, whilst the inclusions were left colourless. It was later found possible to differentiate between them by the use of carbol fuchsin differentiated with picric acid. The nucleolus then took a red stain whilst the inclusion became yellow. The nucleolus and the crystal also responded somewhat differently to acid fuchsin by whatever method it was applied or differentiated.

DISCUSSION.

The Cytoplasmic Inclusions.—The amorphous inclusions of severe etch show many points of similarity with those of aucuba mosaic virus (Bawden and Sheffield, 1939). Their mode of formation by the aggregation of particles appears to be identical. Both are obviously mixtures of chemically different materials. Each contains a large proportion of a protein constituent, and chondriosomes and fats are present in both, but the severe etch bodies appear to contain a rather larger proportion of fats. Both disintegrate if suspended in media of lower osmotic pressure than that of the cells from which they are extracted. Both contain virus, but in both cases virus is present also in the rest of the cell.

The numbers of lesions given by a 1 in 10^8 or 1 in $2 \cdot 10^7$ dilution of aucuba mosaic bodies is comparable to the numbers of lesions given by similar dilutions of purified virus. But with severe etch virus few infections can be obtained with expressed sap at a dilution greater than 1 in 10^3 , but a suspension of inclusions gives infections even when diluted to 1 in 10^8 . The amount of virus contained in expressed sap of hosts infected with severe etch is many times less than that obtained if the infection is with aucuba mosaic. But the results obtained with the inclusions suggest that comparable weights of the two viruses may produce comparable amounts of infection. It may not be that less virus is produced in hosts infected with severe etch but possibly it is in such a form that it is not so readily available.

As the inclusions are infective they must contain virus, and as they do not give Feulgen's reaction it seems that if severe etch virus is a nucleoprotein, like the other viruses isolated, it must also contain nucleic acid of the yeast type. Unfortunately there is no simple colour test known for nucleic acid of this type.

Bodies induced by aucuba mosaic are isotropic whereas those of severe etch virus contain minute doubly refractive particles which in some cases are in active Brownian movement within the body. The inclusions of both viruses tend to crystallize. The aucuba mosaic bodies usually give hexagonal crystals, but sometimes give needle-like forms of 20 or more microns in length. The severe etch bodies may give birefringent needles which are smaller, being only $2 \cdot 5\mu$ in length. Both types of inclusion tend to disappear from older

cells. Both may become vacuolate, the aucuba mosaic body soon after formation but the severe etch body only in the process of dissolution.

The severe etch bodies are less compact than those of aucuba mosaic, which behave as if they are bounded by a surface membrane. This is suggested by their appearance, by their behaviour in weak alkalis, where swelling occurs prior to shrinkage, and in their behaviour on pricking, when a vesicle may be formed prior to complete dissolution. The severe etch bodies can be pricked or divided without any apparent effect. In this they are similar to the bodies of Hy. III virus, to which severe etch shows many affinities. The amorphous bodies of the L strain of potato virus X, which take an amœboid rather than a granular form, can be similarly divided into portions without causing general disintegration.

As to the nature of the amorphous bodies of severe etch virus, it is at present only possible to reiterate the suggestion made as to the nature of the aucuba mosaic bodies, i.e. that the protein constituent consists of virus possibly in combination with some other cell constituent either normally present or produced as a result of infection. Differences between the physical properties of the bodies of the two diseases might be accounted for by those slight chemical differences in the structure of the viruses which confer on them their differing properties. The relatively larger number of bodies produced by severe etch virus might account in part for the apparent lower concentration in expressed sap. Virus in the inclusions would be less readily extracted than that which may be suspended in the cell sap.

The Nuclear Inclusions.—In considering these inclusions two questions immediately spring to mind. Firstly, what is the relationship between these bodies and other structures normally present in the cell, especially those within the nucleus? Secondly, what is the relationship between the crystals and the virus?

The foregoing experiments have shown that although both contain protein the chromosomes are basophilic in their staining reactions whilst the crystals are acidophilic like the nucleoli. But they show some differences in staining capacity from the nucleoli. They are not formed in connection with or at the expense of the nucleoli.

In many ways these intranuclear inclusions seem to parallel closely the crystalline inclusions which have been described as occurring in normal plants. Tischler (1934) has listed and discussed these. His list contains more than 100 species, comprising many genera among the Pteridophyta and Gymnospermæ as well as dicotyledons and monocotyledons. None has been described among healthy plants of the Solanaceæ.

These crystals are dissolved by pepsin and give protein colour reactions. They are insoluble in water. As to their solubilities in acids, alkalis, and alcohol, there is no agreement, probably due to the existence of different solubilities in the inclusions of different species. They appear to be related to the nucleoli from which they can be distinguished by staining reactions. If preparations are stained in pure acid fuchsin and then washed in water

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the dye leaves the nucleolus whilst the crystal is still red. The crystals of plants infected with severe etch behave similarly. In healthy plants a double stain of Delafield's hæmatoxylin and acid fuchsin results in purple nucleoli and red crystals. This has not yet been applied to virus-infected material. In some cases (e.g. *Galtonia*) large crystals accompany small nucleoli, and vice versa. In *Dahlia variabilis* the nuclei contain either a nucleolus or a crystal. One of the guard cells of a stoma may contain a crystal whilst the other has a nucleolus in its nucleus. These crystals are elongated and may project from the nucleus.

At nuclear division the crystals may dissolve within the nucleus or may pass into the cytoplasm, where they dissolve. This is similar to the behaviour of the crystals induced by severe etch virus at the abnormal divisions which may occur after their formation.

In Tischler's list *Ligustrum vulgare* is quoted as containing protein crystals in the nuclei. For comparison with the virus bodies the writer examined some species of *Ligustrum*. In the ordinary green varieties of *L. vulgare* and *L. ovalifolium* no crystals were found, but in a variety of golden privet they were present in every cell of the leaf. They were tetrahedral and smaller and fewer in number than those induced by severe etch virus. Their apparent absence from the green and prevalence in the golden variety suggested a possible connection between them and the occurrence of chlorosis. Especially as some variegations in privet can be transmitted by grafting (Baur, 1904, '06, '07) it is felt that the subject should be more fully investigated before further comment is made.

Intranuclear inclusions also occur in many animal tissues (Findlay, 1938). Those which accompany virus diseases have been classified according to whether they cause complete degeneration of the nucleus or whether their effect is localized. The polyhedral diseases of insects have been classified as a third group. The bodies produced by them are more angular in form and bear a certain similarity in shape and staining reactions to the severe etch bodies. But in the latter there is no apparent disturbance of the nuclear contents and the subsequent hypertrophy which occurs when inclusions are formed in the polyhedral diseases. As in plants, animal virus bodies can be differentiated from the chromatin by use of Feulgen's reagent (Ludford, 1930).

Intranuclear inclusions arise in animals from causes other than infection with virus diseases and they have been produced experimentally.

Intranuclear inclusions are seen to be fairly widespread in both plant and animal kingdoms, but no very satisfactory explanation of their occurrence has been offered. It is, of course, possible that they arise from a variety of causes. In the case of the virus bodies it is tempting to suggest that they essentially contain virus by analogy with the amorphous inclusions which have been shown to be infective. In support of this the crystals induced by severe etch virus have many staining properties in common with one component of the amorphous bodies. There is, however, no evidence that the intranuclear inclusions are infective. The polyhedra, which are the

only ones with which satisfactory results have been obtained, are definitely not infective (Glaser, 1928).

Tischler (1934) suggests that the protein crystals in plant nuclei may have an ecological significance, but he inclines to the view that they are a reserve substance. For this view there is a certain amount of support. They tend to be absent from older tissues. In one case they were present in the nucellus but dissolved later. They are present in the embryo of the ungerminated seed of *Mirabilis*, but as it germinates they first swell, but then fragment and dissolve. On the other hand, they are often still present in very old tissues, and in bud scales, even after shedding, and in plants which have been kept in the dark.

There is some evidence that they are due to an unbalanced metabolism. Besides their occurrence in plants infected with severe etch virus and their presence in a variegated but not in green varieties of *Ligustrum*, we know that in *Pelargonium* they may be induced by the presence of *Bacterium tumefaciens*. In *Fraxinus* their presence may lead to hypertrophy and subsequent degeneration of the nuclei: a process unlikely to occur in completely healthy tissue. Intranuclear crystals were found in a species of *Ceratium* growing in the Bay of Naples, but this same species produced no inclusions when growing in the Kiel Canal. This suggests a possible diseased condition of one group of plants. Most of the observations on intranuclear crystals in normal plants were made prior to the present century. Some of the work might with advantage be repeated, for in the light of more recent knowledge on the physiological requirements of the plant as well as on the existence and nature of diseases with an ætiological agent, it might be found that the examples cited were not in a completely normal condition. A fault might lie in their nutrition, or they might be suffering from an attack from some parasite, or they might be carrying a latent virus which showed no external symptoms. Any such causes would result in an unbalanced metabolism.

SUMMARY.

Severe etch virus induces two types of intracellular inclusion.

The cytoplasmic inclusions are amorphous. Chemically they consist of mixtures of proteins with fats and lipoids. They are formed by the aggregation of particles which appear in the streaming cytoplasm. They may contain some birefringent particles and may give rise to small needle-like bodies. They can be pricked or divided into portions with a microneedle. They contain the virus, but this is also present in other parts of the cell. They are numerous and occur in most tissues of the plant.

The intranuclear inclusions give protein reactions and are more stable than the cytoplasmic inclusions. They take the form of thin rectangular plates, and as many as 30 may be found in a single nucleus. They can be isolated but can be broken or dissolved only with difficulty. Almost every nucleus contains them and they have been found in almost all tissues. They

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occur in the seed but have not been found in the young embryo. The virus is not transmitted through the seed.

The two types of inclusion are briefly discussed and compared with other inclusions occurring in diseased and healthy tissues.

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DESCRIPTION OF PLATES.

Unless it is otherwise stated all photographs are from tobacco infected with severe etch virus.

All photographs were taken with a Leitz Makam camera. The source of illumination was either a mercury vapour lamp or a Leitz Monla lamp. Fixatives, stains, source of illumination, Wratten colour filters and magnifications are given in that order after the description of each figure.

PLATE I.

- Fig. 1.—Part of a hair cell from the surface of a mottled leaf. The nucleus containing several crystals lies against the wall. Small particles are seen in the cytoplasmic strands. At one junction of strands some particles are accumulating. (Living, unstained, mercury vapour, 62, $\times 450$.)
Figs. 2 and 3.—Part of a hair cell at a later stage of infection. The nucleus contains many crystals and the cytoplasmic inclusion is fully formed. (Technique as for fig. 1.)
Fig. 4.—Part of hair cell from deformed leaf. Crystals are present in the nucleus. Minute needle-shaped bodies are apparent in the amorphous inclusion. (Living, unstained, Monla, B and 22, $\times 450$.)
Fig. 5.—Part of epidermal cell from beneath vein of deformed leaf. Crystals are present in the nucleus. Minute needle-shaped bodies are appearing in the cytoplasmic inclusion. (Technique as fig. 4.)
Fig. 6.—As fig. 5. The cytoplasmic inclusions are becoming vacuolate. (Technique as figs. 4-5.)

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- Fig. 7.—Meristematic tissue of a young leaf. Several nucleoli have appeared in each nucleus. Thin rectangular crystals are also forming. Some are seen flat and others in edge view. (Champy, Kull, mercury vapour, 62, $\times 900$.)
- Fig. 8.—Very slightly later stage than fig. 7. Infection has induced a further nuclear division and one cell is seen in telophase. A crystal has been thrown out of this nucleus and is seen in edge view below the group of chromosomes on the right. Other nuclei contain many nucleoli and some contain small crystals. (Technique as fig. 7.)

PLATE II.

- Fig. 1.—Nuclear division is induced in already differentiated cells. That shown in this figure was taken from the ground tissue near a vascular bundle. A crystal which was formed in the parent nucleus is seen to the left of the mitotic figure. (Champy, Kull, mercury vapour, 62, $\times 900$.)
- Fig. 2.—Nuclear division in a palisade cell has not been followed by cell division so that a cell now contains two nuclei. A crystal formed in the parent nucleus is now seen in the cytoplasm. Other nuclei contain nucleoli and also small crystals in flat and edge views. (Technique as fig. 1.)
- Fig. 3.—Nucleus showing differential staining of nucleolus and crystal; the former is red, the latter colourless. (Champy, pyronin, mercury vapour, 62, $\times 900$.)
- Fig. 4.—The osmic impregnation method leaves the crystals and nucleoli brown in colour. Small dark staining particles may be included within the crystals. (Champy, Kolachev, mercury vapour, 62, $\times 900$.)
- Fig. 5.—The osmic impregnation method results in much of the material of the amorphous body taking a black stain. (Technique as fig. 4.)
- Fig. 6a.—A strip of epidermis from beneath the leaf vein shows intranuclear and cytoplasmic inclusions. (Living, unstained, Monla, B and 22, $\times 450$.)
- Fig. 6b.—The same field as fig. 6a seen between crossed Nicol prisms. By comparison with fig. 6a crystals in edge view are seen to be birefringent; one which is lying flat (top, right corner) is isotropic. The amorphous bodies contain doubly refractive particles (to left and lowermost cells). Other "flares" seen in fig. 6b are due to secreted material lying on the leaf surface. (Living, unstained, Monla, polarized light, $\times 450$.)

PLATE III.

- Figs. 1a-d.—Inclusion of aucuba mosaic virus from *Solanum nodiflorum*. This inclusion was isolated together with the nucleus in a 0.1 molar solution of phosphate buffer at pH 8 (fig. 1a). After 10 minutes it had swollen considerably (fig. 1b). Then the nucleus passed inside the body, which began to shrink. (Fig. 1c was taken 30 minutes after isolation.) After 1 hour no further shrinkage occurred (fig. 1d). The amorphous inclusions of severe etch virus behave similarly in alkaline solution. (Living, unstained, mercury vapour, unscreened, $\times 450$.)
- Figs. 2a-c.—Inclusions of aucuba mosaic virus from *S. nodiflorum*. Neither these inclusions nor those of severe etch show any change in weak acids. This inclusion was isolated into a phthalate buffer solution at pH 3.4 and 0.1 molarity (fig. 2a). No change was seen after 3 hours (fig. 2b) or 48 hours (fig. 2c). (Technique as fig. 1.)
- Fig. 3.—The cytoplasmic inclusions of severe etch virus are unaffected by pricking. (Technique as fig. 1. The black outline is due to the hair having become partially immersed in air.)
- Fig. 4.—The cytoplasmic inclusions can be divided into portions. (See fig. 3.)
- Fig. 5.—If crushed the inclusions break into particles but do not dissolve. (Technique as fig. 1.)

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EFFECT OF HEAT ON FLOCCULATING ANTIBODIES OF RABBIT ANTISERA.

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EARLIER workers have found that antisera to different antigens vary in their resistance to heat. In general it has been found that bacterial somatic agglutinins lose their flocculating power with less heating than flagellar agglutinins. For example, Jones (1927) showed that flagellar agglutinin to hog-cholera bacillus still agglutinated after the antiserum was heated at 90° C. for 20 minutes, whereas somatic agglutinin did not after the same time at 75° C. It has been also shown that some antisera which have lost their ability to agglutinate after heating for several minutes at 70-80° C. still combine with bacteria to give the phenomenon of inhibition (Eisenberg and Volk, 1902; Jones, 1928). This ability to inhibit is destroyed at 90° C., i.e. approximately the temperature needed for destruction of flagellar agglutinins.

Bawden and Pirie (1938*b*) showed that plant viruses with rod-shaped particles form with their antisera fluffy, open floccules similar to those formed by the agglutination of bacterial flagellar antigens, whereas those with spherical (or almost spherical) particles form dense granular precipitates similar to those formed by the agglutination of bacterial somatic antigens. When antisera to these two types of viruses are heated, they behave in the same way as antisera to the two types of bacterial antigens. Strong antisera to rod-shaped tobacco mosaic virus flocculate until heated for 10 minutes at 90° C., whereas antisera to the spherical tomato bushy stunt virus do not flocculate after heating for 10 minutes at 75° C. This paper describes experiments made to investigate the causes underlying the differences in the apparent behaviour of different antisera on heating.

METHODS AND MATERIAL.

The antigens used were human serum globulin and albumin, a strain of pea nodule bacteria (*Rhizobium leguminosarum*) and purified preparations of the following plant viruses: tobacco mosaic, potato "X," tomato bushy stunt and tobacco necrosis.

The preparations of plant viruses were made by precipitation methods and kindly supplied by Mr. F. C. Bawden.

Human globulin was prepared by half saturating human serum with ammonium sulphate. The precipitate was dissolved in water and dialysed. NaCl was then added up to 0.9 per cent. to dissolve the precipitate formed during dialysis. Albumin was precipitated by full saturation with ammonium sulphate of the filtrate remaining after the precipitation of globulin. This precipitate was dissolved in water and dialysed, and NaCl to 0.9 per cent. added.

Suspensions of pea nodule bacteria were prepared by washing the cultures grown on agar slopes with 0.9 per cent. NaCl. The bacteria were then killed with 1 per cent. formaldehyde and washed with NaCl solution.

The antisera were prepared by injecting rabbits intravenously twice a week with solutions or suspensions of the antigens. From 4 to 6 injections in all were given and the rabbits were bled 8-10 days after the last.

Most of the antibodies in the antisera to all these antigens precipitated out with the euglobulin fraction. These fractions were precipitated with 1/3 saturated ammonium sulphate. The precipitate was filtered off, dissolved in 0.9 per cent. NaCl and dialysed against 0.9 per cent. NaCl solution until the test for SO_4^{2-} with BaCl_2 was negative.

The fractions of normal rabbit serum used in this work and the methods of heating were the same as those described in the previous paper (Kleczkowski, 1941).

Flocculation tests were made by mixing 1 ml. of antigen solutions with either 1 ml. of antiserum solutions or 1 ml. of antiserum euglobulin solutions. The tubes were immediately placed in a water bath at 50° C. The appearance of floccules was taken as a positive result. The ability of heated solutions to combine with antigen without causing flocculation was tested by their ability to inhibit flocculation. The solutions to be tested were mixed with

antigen solutions and incubated for 3 hours at 50° C., when 0.1 ml. of a suitable dilution of unheated antiserum was added. The absence of flocculation was taken as evidence of inhibition.

RESULTS.

The effect of heating antisera to tobacco mosaic virus and tomato bushy stunt virus.

The results shown in Table I illustrate differences in the behaviour of tobacco mosaic virus and bushy stunt virus antisera after heating. The precipitating power of bushy stunt antiserum is completely destroyed by heating for 10 minutes at 75° C., but the ability to combine with antigen is preserved; this is shown by the inhibition of flocculation when unheated antiserum is subsequently added. The inhibition is specific; it is not caused by incubation with either heated normal serum or heated heterologous antisera. The inhibition is more pronounced after heating for 10 minutes at 80° C. (higher "inhibition titre") and it still occurs after heating for 10 minutes at 85° C. It is destroyed by heating for 10 minutes at 90° C.

TABLE I.—*The Effect of Heating Antisera to Tobacco Mosaic and Bushy Stunt Viruses for 10 Minutes at Different Temperatures.*

The antisera were heated at a dilution 1/10 in physiological saline.

The antigen solutions were used at 0.0025 per cent. for tobacco mosaic virus and 0.005 per cent. for bushy stunt virus. 1 ml. of antigen solution was added to a series of tubes each containing 1 ml. of antiserum at varying dilutions. + indicates precipitation and — no precipitation.

After 3 hours 0.1 ml. of control antiserum at a dilution 1/8 was added to the tubes where there was no precipitation. i indicates that there was still no precipitation (inhibition), and o that a precipitate was formed (no inhibition).

Temperature of heating.	Dilution of the antiserum.														
	Tobacco mosaic virus.							Bushy stunt virus.							
	1/10.	1/20.	1/40.	1/80.	1/160.	1/320.	1/640.	1/10.	1/20.	1/40.	1/80.	1/160.	1/320.	1/640.	
Unheated	+	+	+	+	+	+	—	+	+	+	+	+	+	+	—
							o								o
75° C.	+	+	+	+	+	—	—	i	i	i	i	o	o	o	—
						o	o	—	—	—	—	—	—	—	—
80° C.	+	+	+	—	—	—	—	i	i	i	i	i	o	o	—
				o	o	o	o	—	—	—	—	—	—	—	—
85° C.	—	—	—	—	—	—	—	i	i	o	o	o	o	o	—
	o	o	o	o	o	o	o	—	—	—	—	—	—	—	—
90° C.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o

By contrast, the precipitating power of tobacco mosaic virus antiserum is only slightly diminished by heating for 10 minutes at 75° C., and further diminished, but not destroyed, by heating at 80° C. Sera with higher titres are only inactivated after 10 minutes at 90° C. Thus there are two obvious differences in the behaviour of the two antisera. The bushy stunt antiserum

loses its ability to precipitate at a lower temperature than tobacco mosaic antiserum, but it then inhibits precipitation, whereas no such phenomenon can be observed with the heated tobacco mosaic antiserum. The amount of heat necessary to inactivate tobacco mosaic antiserum is apparently the same as that required to destroy the ability of bushy stunt virus antiserum to combine with antigen.

The influence of dilution of antisera in saline and in protein solutions on the effect of heating.

The results of experiments given in Tables II and III show that diluting antisera to tobacco mosaic and bushy stunt viruses in saline lessens the destructive effect of heating on their precipitating power; with bushy stunt virus antiserum it also prevents the appearance of the phenomenon of inhibition. On the other hand, diluting the antisera in protein solutions (normal rabbit serum, rabbit serum albumin and pseudoglobulin, human serum albumin) enhances the destructive effect of heating on the precipitating power and with bushy stunt antiserum it also enhances the appearance of inhibition phenomenon. The inhibition is stronger after diluting in albumin solutions than after diluting in whole rabbit serum or in pseudoglobulin solution.

TABLE II.—*The Effect of Heating Tobacco Mosaic Virus Antiserum Diluted in Physiological Saline and in Normal Rabbit Serum.*

The antiserum was heated for 10 minutes at 75° C. and 80° C. at varying dilutions in saline and in normal rabbit serum diluted 1/10 in saline.

Dilution.	Diluent.	Temperature of heating.	Dilution of antisera.				
			1/50.	1/100.	1/200.	1/400.	1/800.
—	Saline	Unheated.	+	+	+	+	—
1/10	„	75° C.	+	+	+	—	—
1/50	„	„	+	+	+	+	—
1/50	Serum	„	+	+	—	—	—
1/100	Saline	„	..	+	+	+	—
1/100	Serum	„	..	+	—	—	—
1/10	Saline	80° C.	—	—	—	—	—
1/50	„	„	+	+	—	—	—
1/50	Serum	„	—	—	—	—	—
1/100	Saline	„	..	+	—	—	—
1/100	Serum	„	..	—	—	—	—

Symbols as in Table I.

TABLE III.—*The Effect of Heating Bushy Stunt Virus Antiserum Diluted in Physiological Saline and in Protein Solutions.*

The antiserum was heated for 10 minutes at 75° and 80° C. at varying dilutions in saline, in normal rabbit serum diluted 1/10 in saline and in 0.6 per cent. solutions in saline of rabbit serum albumin, pseudoglobulin or human serum albumin.

Dilution.	Diluent.	Temperature of heating.	Dilution of antisera.				
			1/50.	1/100.	1/200.	1/400.	1/800.
..	Saline	Unheated	+	+	+	+	+
1/10	..	75° C.	—	—	—	—	—
1/50	+	o	o	o	o
1/50	Rabbit serum	..	—	o	o	o	o
1/100	Saline	+	—	—	—
1/100	Rabbit serum	—	—	—	—
1/10	Saline	80° C.	—	+	+	+	+
1/100	—	—	—	—
1/100	Rabbit serum	o	o	o	o
1/100	Rabbit albumin	—	—	—	—
1/100	Rabbit pseudoglobulin	—	—	—	—
1/100	Human albumin	—	—	—	—

Symbols as in Table I.

Streng (1909) and earlier workers showed that diluting bacterial antisera in saline slows down the heat inactivation of agglutinins and also prevents the appearance of the inhibition phenomenon. Diluting in physiological saline is one of the means of preventing coagulation, i.e. the formation of large complexes of protein particles, which follows denaturation. Other means of preventing coagulation, such as varying pH or addition of substances like urea, etc., also reduce the rate at which heat causes antisera to lose their flocculating power (Marrack, 1938). These facts suggest that loss of the flocculating power of antibodies, or the exchange of this power for the ability to inhibit after heating, is a result of production of large complexes of protein particles. The fact that the addition of unspecific proteins before heating has an effect similar to using more concentrated antiserum solutions suggests that the complexes containing antibody are produced by the union of different protein fractions. They are formed during heating, for the addition of unspecific proteins after heating has no such effect. The possibility of unspecific proteins affecting the behaviour of antisera on heating was indicated by Pick (1902) (quoted by Streng, 1909), who showed that typhus agglutinins "purified" by salting out (i.e. when a considerable amount of unspecific protein, like

albumin, had been removed) were much less affected by heating than the original antiserum.

The experiments described below were made to test these conclusions and to gain additional information about the role played by unspecific proteins during heating of antisera.

The effect of heating euglobulin fractions of antisera separately and in the presence of other fractions.

The rate of destruction of flocculating power by heat is much slower in the case of euglobulin fractions of antisera than with either whole antisera or mixtures of euglobulin fractions and albumin.

Tables IV and V illustrate this for tobacco mosaic virus antibodies. The difference can be seen when the solutions are heated for varying lengths of time at 70° C. (Table IV), but it is much more definite when they are heated at 75° C. (Table V). The precipitation titre of the whole antiserum and of its euglobulin fraction heated in the presence of albumin falls from 1/640 and 1/160 respectively to less than 1/10 within 20 to 40 minutes, whereas the titre of euglobulin heated alone is almost unaffected after 40 minutes and is only reduced from 1/160 to 1/40 after 160 minutes.

TABLE IV.—*The Effect of Heating at 70° C. for Varying Lengths of Time Tobacco Mosaic Virus Antiserum and Euglobulin Fraction of the Antiserum in the Presence and Absence of Rabbit Serum Albumin.*

The antiserum was heated at a dilution 1/10 in saline. The euglobulin fraction of the antiserum (protein concentration 0.6 per cent.) was heated at a dilution of 1/10 in saline and in 0.4 per cent. rabbit albumin solution in saline.															
Preparation of antiserum.	Time of heating (in minutes).	Dilution of the antiserum or of the euglobulin fraction.													
		1/80.	1/100.	1/120.	1/140.	1/160.	1/200.	1/240.	1/280.	1/320.	1/400.	1/480.	1/560.	1/640.	1/800.
Whole antiserum	0	+	+	+	+	—
	15	+	+	+	+	+	—	—	..
	30	+	+	+	+	+	—	—	—	..
	60	+	+	+	+	—	—	—	—	..
	120	+	+	+	+	+	+	—	—	..
	240	+	+	+	+	—	—	—	—	..
Euglobulin diluted in saline	0	+	+	+	+	—									
	15	+	+	+	+	—									
	30	+	+	+	—	—									
	60	+	+	+	—	—									
	120	+	+	+	—	—									
	240	+	+	—	—	—									
Euglobulin diluted in albumin.	0	+	+	+	+	—									
	15	+	+	+	—	—									
	30	+	+	—	—	—									
	60	+	+	—	—	—									
	120	+	+	—	—	—									
	240	+	+	—	—	—									

Symbols as in Table I. Only the results of precipitation are recorded. There was no inhibition.

TABLE V.—*The Effect of Heating at 75° C. for Varying Lengths of Time Tobacco Mosaic Virus Antiserum and the Euglobulin Fraction of the Antiserum in the Presence and Absence of Rabbit Serum Albumin.*

The solutions used for heating were as described in Table IV.

Preparation of antiserum.	Time of heating (in minutes).	Dilution of the antiserum or of the euglobulin fraction.							
		1/10.	1/20.	1/40.	1/80.	1/160.	1/320.	1/640.	1/1280.
Whole antiserum	0	+	+	+	+	+	+	+	+
	10	+	+	+	+	+	+	—	—
	20	—	+	+	+	—	—	—	—
	40	—	—	—	—	o	o	o	o
	80	—	—	—	—	—	—	—	—
	160	—	—	—	—	—	—	—	—
		o	o	o	o	o	o	o	o
Euglobulin diluted in saline	0	+	+	+	+	+	—	—	—
	10	+	+	+	+	+	o	—	—
	20	+	+	+	+	+	—	—	—
	40	—	+	+	+	+	o	—	—
	80	—	+	+	+	—	—	—	—
	160	+	+	+	—	—	—	—	—
					o	o	o		
Euglobulin diluted in albumin	0	+	+	+	+	+	—	—	—
	10	+	+	+	—	—	o	—	—
	20	—	—	—	o	—	—	—	—
	40	—	—	—	—	—	—	—	—
	80	—	—	—	—	—	—	—	—
	160	—	—	—	—	—	—	—	—
		i	i	i	o	o	o		

Symbols as in Table I.

Table V shows inhibition as a result of heating tobacco mosaic virus antiserum. The effect is slight compared with that of bushy stunt virus (compare Tables I and VI) and it is only transient. In the mixture of euglobulin with albumin, however, the production of inhibition is much more definite and lasting, although still less than with bushy stunt virus. In the mixture the ratio albumin/globulin is 7/1, i.e. more than three times greater than in antiserum.

The presence of protein fractions other than euglobulin in the heated

solutions affects bushy stunt virus antibodies much more than tobacco mosaic virus antibodies. Table VI shows that heating at 70° C. is here sufficient to demonstrate considerable differences. The precipitating power of the euglobulin fraction of bushy stunt virus antiserum heated alone is but little affected

TABLE VI.—*The Effect of Heating at 70° C. for Varying Lengths of Time Bushy Stunt Virus Antiserum and the Euglobulin Fraction of the Antiserum in the Presence and Absence of Rabbit Serum Albumin.*

The antiserum was heated at a dilution 1/10 in saline. The euglobulin fraction of the antiserum (protein concentration 0.75 per cent.) was heated at a dilution 1/10 in saline and in 0.4 per cent. rabbit albumin solution in saline.

Time of heating (in minutes).	Dilution of whole antiserum.								Dilution of the euglobulin fraction heated alone.							
	1/10.	1/20.	1/40.	1/80.	1/160.	1/320.	1/640.	1/1280.	1/10.	1/20.	1/40.	1/80.	1/160.	1/320.	1/640.	1/1280.
0	+	+	+	+	+	+	+	—	+	+	+	+	+	+	+	—
15	+	+	+	+	+	+	—	—	+	+	+	+	+	+	+	—
30	+	+	+	+	+	—	—	—	+	+	+	+	+	+	+	—
60	+	+	+	—	—	—	—	—	+	+	+	+	+	+	+	—
120	+	+	—	—	—	—	—	—	+	+	+	+	+	+	+	—
240	+	—	—	—	—	—	—	—	+	+	+	+	+	+	+	—
		i	i	i	i	o	o	o								o

Dilution of the euglobulin fraction heated in presence of rabbit serum albumin.																
	1/10.	1/25.	1/45.	1/17.5.	1/20.	1/25.	1/30.	1/35.	1/40.	1/80.	1/160.	1/200.	1/240.	1/280.	1/320.	1/400.
0	+	+	+	+	+	+	o
15	+	+	+	+	+	+	+	—
30	+	+	+	+	+	—	—	—
60	+	+	+	+	—	—	—	—	—	..
120	+	+	+	—	—	i	i	o	o	..
240	+	—	—	—	—	—	—	—	—	..
		i	i	i	i				i	i	i				o	

Symbols as in Table I.

after 240 minutes, whereas the precipitation titre of the whole antiserum and of its euglobulin fraction heated in the presence of albumin falls from 1/640 and 1/320 respectively to 1/10. With the fall in the precipitation titre the phenomenon of inhibition appears. It first appears after about 60 minutes and its zone increases with the time of heating. It is definite before the precipitating power of heated solutions is totally destroyed. In such cases

precipitation occurs in more concentrated antibody solutions and inhibition in the more dilute solutions.

When bushy stunt virus antiserum is heated at 75° C., its precipitating power is completely destroyed within 10 minutes and is replaced by inhibition (Table I), and the euglobulin fraction of the antiserum heated with 0.4 per cent. of albumin behaves similarly. But when the euglobulin fraction is heated alone, its precipitating power is almost unaffected after 10 minutes, and is not much decreased after 160 minutes (Table VII). However, a zone of inhibition appears after 10 minutes of heating; this looks like an antibody excess zone, and it narrows as the time of heating increases.

TABLE VII.—*The Effect of Heating at 75° C. for Varying Lengths of Time the Euglobulin Fraction of Bushy Stunt Virus Antiserum.*

The euglobulin fraction (protein concentration 0.75 per cent.) was heated at a dilution 1/10 in saline.

Time of heating.	Dilution of the euglobulin fraction.						
	1/10.	1/20.	1/40.	1/80.	1/160.	1/320.	1/640.
0	+	+	+	+	+	+	o
10	— i	— i	+	+	+	+	— o
20	— i	— i	+	+	+	+	— o
40	— i	+	+	+	+	—	o
80	— i	+	+	+	+	—	— o
160	+	+	+	+	+	—	o

Symbols as in Table I.

Evidence has previously been given suggesting that normal rabbit serum proteins, while undergoing heat denaturation (in presence of 0.9 per cent. NaCl at pH near neutrality), combine with one another to form large mixed complexes (Kleczkowski, 1941). The results obtained with heated antisera suggest that antibodies can also unite with other proteins to form complexes. The serological behaviour of these complexes is determined by the other proteins present in the solution during heating and also by the type of antigen. When antibodies to either virus are heated with euglobulin fractions of the antisera only, they can still unite with and flocculate their antigens. That their properties are altered, however, is shown by slight changes in the precipitation. For example, tobacco mosaic virus precipitates are less nebulous and the floccules appear quicker than with unheated antibodies, and with bushy stunt virus antibodies the range of antibody excess inhibition is increased, so that precipitation is restricted to a narrower range of antigen/antibody ratios.

When antibodies are heated in the presence of protein fractions other than euglobulin much greater changes occur, and the differences between antigens of flagellar (tobacco mosaic) and somatic (bushy stunt) type become definite. The complexes formed in such mixtures of proteins can combine with their

antigens, but cannot cause flocculation, and their combination with antigen can prevent the latter from being precipitated subsequently by unchanged antibody. In a solution containing both changed and unchanged antibodies there is a competition between them, and the flocculation titre is the result of that competition. The titre, therefore, cannot be taken as a direct measure of the amount of antibody which remains unchanged, as authors who previously investigated the problem of the heat inactivation of antibodies have done. The result of the competition depends largely on properties of antigen particles. Consequently, after the same amount of heating in identical conditions tobacco mosaic virus antiserum can still precipitate its antigen, whereas bushy stunt virus antiserum has lost its precipitating power and inhibits. The results of the experiments described below show that such a competition does occur.

Effects of mixing heated and unheated antisera in varying proportions.

Antiserum to bushy stunt virus diluted 1/10 with saline was heated for 20 minutes at 80° C., so that its precipitating power was destroyed and inhibition was pronounced. The heated antiserum was then mixed with varying

TABLE VIII.—*The Effect of Mixing in Varying Proportions Heated and Unheated Bushy Stunt Virus Antiserum.*

The antiserum was heated for 20 minutes at 80° C. at a dilution of 1/10 in saline. This was mixed in varying proportions with unheated antiserum and the mixtures tested as described in Table I, or heated antiserum was first added to the solution of antigen and unheated antiserum followed after 1 hour.

Exp. number.	Ratio of heated antiserum to total amount of antiserum.	Dilution of the total antiserum.						
		1/10.	1/20.	1/40.	1/80.	1/160.	1/320.	1/640.
<i>A. Heated and Unheated Antiserum added Simultaneously.</i>								
1	0.0	.	+	+	+	+	+	+
2	0.2	.	+	+	+	+	+	o
3	0.4	.	+	+	+	—	—	—
4	0.5	.	+	+	—	o	o	o
5	0.6	.	+	—	—	—	—	—
6	0.66	.	—	i	i	i	o	o
7	1.0	.	—	—	—	—	—	—
			1	i	i	i	i	o
<i>Heated Antiserum Mixed first with Antigen Solution and Unheated Antiserum followed after 1 hour.</i>								
8	0.2	.	—	—	+	+	+	—
			i	i	—	—	—	o
9	0.4	.	—	—	—	—	—	—
			i	i	i	o	o	o

Symbols as in Table I.

amounts of unheated antiserum and the precipitating power of the mixtures was tested. The results are shown in Table VIII.

The competing action of changed antibodies is clearly shown. For example, the unchanged antiserum has a precipitation titre of 1/320, whereas a mixture of equal parts of heated and unheated antiserum has a titre of only 1/20, although there is sufficient unchanged antibody to give a titre of at least 1/160. Thus the competing action of the changed antibody has reduced the precipitation titre to one-eighth, showing the error made by taking the titre as a direct measure of the amount of unchanged antibody in the mixture.

Over the whole range of ratios in which heated and unheated antisera were mixed, there is a drop in titre due to the presence of changed antibody. This drop becomes greater with increasing amounts of heated antiserum in the mixture.

The results of heating bushy stunt virus antiserum for varying lengths of time at 70° C. agree closely with the results of mixing unheated and heated antiserum in varying proportions; this can be seen by comparing Tables VI and VIII. With increasing time of heating at 70° C. (Table VI), as with increasing ratio of heated antiserum to the total amount of antiserum in the mixtures (Table VIII), the precipitation titre decreases. When the titre falls to about one-eighth of the original value the inhibition phenomenon appears, and the zone of inhibition widens with increasing time of heating or with increasing the ratio of heated to total antiserum. In both types of experiment inhibition occurs in the higher antiserum dilutions, while precipitation is still obtained in less dilute solutions. In less dilute solutions the concentration of unchanged antibody is still sufficient to cause precipitation, but in higher dilutions it is insufficient, and the competing action of changed antibody gives complete inhibition.

This similarity between the results shown in Tables VI and VIII suggests that during heating the ratio of changed to unchanged antibody increases, and that the fall in precipitation titre is an indication of this ratio.

Table VIII also shows that the addition of heated antiserum to antigen solution followed by unheated antiserum may give a result different from that when heated and unheated antisera are added simultaneously. Exps. Nos. 8 and 9 correspond to Nos. 2 and 3 respectively, except that in Nos. 8 and 9 unheated antiserum was added 1 hour after the heated antiserum, whereas in Nos. 2 and 3 heated and unheated antisera were added simultaneously. In some tubes the addition of the mixture gave precipitation, whereas there was inhibition when heated antiserum was added before the unheated. This difference again indicates competition between unchanged and changed antibodies. If enough changed antibody has already combined with antigen, unchanged antibody subsequently added cannot cause precipitation. However, if both kinds of antibody are added simultaneously, sufficient unchanged antibody may unite with antigen to give precipitation.

Similar experiments were made with tobacco mosaic virus antiserum and the results are shown in Table IX. Over the range of ratios of unheated to total antiserum from 0.0 to about 0.8 (Exps. Nos. 1-7) the precipitation titres are as they would be without any heated antiserum added, so that changed antibody present in heated antiserum has no visible influence on the precipitat-

TABLE IX.—*The Effect of Mixing in Varying Proportions Heated and Unheated Tobacco Mosaic Virus Antiserum.*

Methods as described for bushy stunt virus antiserum in Table VIII. Only the results of precipitation are recorded. There was no inhibition.

Exp. number.	Ratio of heated antiserum to total amount of antiserum.	Dilution of the total antiserum.							
		1/10.	1/20.	1/40.	1/80.	1/160.	1/320.	1/640.	
<i>A. Heated and Unheated Antiserum Added Simultaneously.</i>									
1	. 0.0	.	+	+	+	+	+	+	—
2	. 0.2	.	+	+	+	+	+	+	—
3	. 0.4	.	+	+	+	+	+	—	—
4	. 0.5	.	+	+	+	+	+	—	—
5	. 0.6	.	+	+	+	+	+	—	—
6	. 0.7	.	+	+	+	+	—	—	—
7	. 0.8	.	+	+	+	—	—	—	—
8	. 0.9	.	—	—	—	—	—	—	—
<i>B. Heated Antiserum in the Exp. No. 8 Substituted by Equal Volume of Similarly Treated Normal Rabbit Serum.</i>									
9	. 0.9	.	+	+	—	—	—	—	—
<i>C. Heated Antiserum Mixed First with Antigen Solution and Unheated Antiserum followed after 1 Hour.</i>									
10	. 0.6	.	+	+	+	+	+	—	—
11	. 0.8	.	—	+	—	—	—	—	—

Methods of testing as in Table I.

ing action of unchanged antibody. However, when the ratio of heated to total antiserum is greater than 0.8 the effect of heat-changed antibody becomes apparent. In Exp. No. 8 there is no precipitation, although the amount of unchanged antibody is sufficient to give a titre of 1/20. This effect is due to the competing specific action of heated antiserum, for the presence of heated normal rabbit serum has no effect on precipitation (Exp. No. 9). Thus, unless heating is prolonged, for most purposes the titre of heated tobacco mosaic virus antiserum is a direct measure of the amount of unchanged antibody after heating.

Comparing Exps. Nos. 5 and 7 with Nos. 10 and 11 (Table IX) gives clearer evidence that heat-changed antibody can combine with tobacco mosaic virus without causing precipitation. The same amounts of heated and unheated antiserum were added to antigen in Exps. Nos. 5 and 10 and in Nos. 7 and 11, but in Nos. 5 and 7 the two were added as a mixture and in Nos. 10 and 11 the heated antiserum was added one hour before the unheated. As with bushy stunt virus, precipitation occurred in some tubes where heated and unheated antiserum were added at the same time, but not when heated antiserum was added first.

Inactivation of inhibiting power.

It has already been shown that heating for 10 minutes at 90° C. destroys

the inhibiting power of bushy stunt virus antiserum (Table I). Heating at lower temperatures for longer periods of time also does this. The inhibition titre at first increases, then remains approximately constant and later decreases. Thus at first there is a production of heat-changed antibodies, which have lost the precipitating power but still possess the ability to combine with antigen, while further heating produces a second change destroying all specific serological activity.

Behaviour of heated antibodies to other antigens.

To determine whether the results obtained with tobacco mosaic and bushy stunt virus antibodies apply at all generally, experiments were made with antibodies to potato virus "X," tobacco necrosis virus, pea nodule bacteria (*Rhizobium leguminosarum*) (317), human serum globulin and albumin.

Antisera to tobacco necrosis virus and potato virus "X" diluted 1/10 in saline were heated for 10 minutes at 80° C. Tobacco necrosis virus antiserum behaved like bushy stunt virus antiserum, the precipitation power being destroyed and replaced by inhibition. Potato virus "X" antiserum, on the other hand, behaved like tobacco mosaic virus antiserum; its precipitation power was not destroyed, the only change being a decrease in the titre and an increased zone of antibody excess inhibition (cf. similar phenomenon with tobacco mosaic antiserum, Table V).

Tobacco necrosis virus, like bushy stunt virus, has quasi-spherical particles (Pirie *et al.*, 1938) and forms specific precipitate of the type "O," whereas potato virus "X," like tobacco mosaic virus, has anisodimensional particles (Bawden and Pirie, 1938a) and forms specific precipitates of the type "H." This indicates that the different results obtained with bushy stunt and tobacco mosaic virus antisera on heating are determined by their different shapes, and that the differences are probably generally applicable to all antigens of somatic ("O") and flagellar ("H") type. This is further supported by the fact that all the additional antigens tested give flocculation of the type "O," and their antisera on heating all behave very like bushy stunt virus antiserum.

Similar experiments were made with bacterial agglutinins. An antiserum to pea nodule bacteria diluted 1/10 in saline and also 0.1 per cent. solution of its euglobulin fraction in the presence and absence of other proteins were heated for 10 minutes at 80° C. The agglutinating power of the antiserum and of its euglobulin fraction heated in the presence of 0.4 per cent. rabbit albumin was destroyed and inhibition was definite, though less pronounced than with bushy stunt or tobacco necrosis viruses. On the other hand, the agglutination titre of euglobulin heated alone fell only from 1/3200 to 1/1600. The presence of 0.4 per cent. pseudoglobulin gave a rather different effect; agglutination was destroyed, but there was no inhibition. When the euglobulin fraction of the antiserum and other fractions were mixed after being heated separately, agglutination occurred just as when the euglobulin was heated and tested separately.

Heating for 10 minutes at 90° C. destroyed both the ability to agglutinate and to inhibit.

TABLE X.—*The Effect of Heating at 70° C. for Varying Lengths of Time Antiserum to Pea Nodule Bacteria and the Euglobulin Fraction of the Antiserum in the Presence and Absence of Rabbit Serum Albumin.*

Preparation of antiserum.	Time of heating (in minutes).	Dilution of the antiserum or of the euglobulin fraction.							
		1/50.	1/100.	1/200.	1/400.	1/800.	1/1600.	1/3200.	1/6400.
Whole antiserum diluted 1/10 in saline	0	+	+	+	+	+	+	+	—
	15	+	+	+	+	+	+	—	—
	30	+	+	+	+	+	+	—	—
	60	+	+	+	+	+	—	—	—
	120	+	+	+	+	+	—	—	—
	240	+	+	+	+	—	—	—	—
0·1 per cent. euglobulin solution in saline	0	+	+	+	+	+	+	—	—
	15	+	+	+	+	+	+	—	—
	30	+	+	+	+	+	+	—	—
	60	+	+	+	+	+	+	—	—
	120	+	+	+	+	+	+	—	—
	240	+	+	+	+	+	+	—	—
0·1 per cent. euglobulin solution in 0·4 per cent. albumin	0	+	+	+	+	+	+	—	—
	15	+	+	+	+	+	—	—	—
	30	+	+	+	+	—	—	—	—
	60	+	+	+	+	—	—	—	—
	120	+	+	+	—	—	—	—	—
	240	+	+	+	—	—	—	—	—

Table X shows the effect of heating the same preparations of bacterial antibodies for varying lengths of time at 70° C. There was no change in the euglobulin fraction heated alone up to four hours, whereas the presence of albumin caused a large fall in the titre; a similar fall occurred when the whole antiserum was heated.

Antisera to both human serum albumin and human serum globulin diluted 1/10 in saline and 0·1 per cent. solutions of euglobulin fractions of the antisera in the presence and absence of 0·5 per cent. rabbit serum albumin were also heated for 15 minutes at 75° C. The precipitating power of whole antisera was destroyed and the phenomenon of inhibition well pronounced. Heating the euglobulin fractions of the antisera alone did not destroy their precipitating power, but when rabbit serum albumin was added to the euglobulin solutions before heating, the precipitating power was destroyed and the inhibition phenomenon was produced. Heating the albumin and the euglobulin separately and then mixing them had no such effect.

DISCUSSION.

Solutions of flocculating (precipitating or agglutinating) antibodies of rabbit antisera twice undergo changes during heating. The first change corresponds with an early stage of denaturation of serum proteins, and coincides with the appearance of active disulphide groups and with the formation of large complexes of changed protein particles (Kleczkowski, 1941). The ability to combine with antigen is not destroyed by this change. The second change corresponds with a further stage of denaturation and coincides with the loss of this ability.

The serological behaviour of antibody after the first change depends on the proteins present in the solution when antibodies are undergoing heat denaturation, for combination between heat-changed antibody particles and those of other proteins seems to occur and the other proteins largely determine the behaviour. Evidence for such a combination between pneumococcal antibody and other proteins has recently been provided by electrophoretic studies on heated horse antisera (Van der Scheer *et al.*, 1941; Krejci *et al.*, 1941).

The name "euglobulin complex" will be used for the complex formed when euglobulin fractions of antisera are heated alone. When other fractions are present, either when whole antisera are heated or when other proteins are added to the euglobulin, "mixed complexes," i.e. composed of particles of different fractions, are formed.

The formation of "euglobulin complexes" only slightly affects the flocculating power of antibody particles. The titre of antibody solutions to both flagellar and somatic types of antigen is affected similarly, and as compared with unheated antibody there are only slight differences in zones of flocculation and type of floccules. It is the formation of "mixed complexes" that is responsible for the wide differences known to exist between the behaviour of heated antisera to somatic and flagellar types of antigen. The "mixed complexes" can still combine with antigens but are unable to cause flocculation. This is true both for flagellar and somatic type of antigens. The difference between the two lies, not, as previously believed, in the difference between the heat stabilities of the antibodies, but in the different competitive effects of unchanged antibody and the "mixed complexes." The precipitating or agglutinating titre of a heated antiserum is a function of this competition, and this is governed by the type of antigen. With anisodimensional antigens, i.e. when the flocculation corresponds to the type "H," the competing action of "mixed complexes" is slight, and the titre of heated antiserum is an approximate measure of antibody which remains unchanged after heating. But with antigens like bushy stunt virus, tobacco necrosis virus, blood serum proteins and bacteria giving "O" type floccules, the "mixed complexes" interfere effectively with the flocculating action of unchanged antibodies and the titre is not a direct measure of the amount of these.

With "O" antigens there are two causes leading to a fall in flocculating titre of antisera after heating, the transformation of antibodies into a form unable to flocculate and the ability of the transformed antibodies to interfere with the flocculating action of unchanged antibodies. With "H" type of antigens, however, the fall in flocculating titre results almost entirely from the first cause. As most tests on heating antisera have been made with "O" type antigens, it is obvious that published work on rate of inactivation of antisera by heat (Streng, 1909; Madsen and Streng, 1910), in which agglutinating titres have been used as a direct measure of antibodies remaining unchanged, needs reconsidering. The use of flocculating titres for this purpose has been responsible for the belief that antibodies to different antigens differed in their resistance to heat, and led Marrack (1938) to suggest that "the destruction of some antibodies must be associated with earliest degrees of heat denaturation of proteins, while others can resist even complete denaturation." All the antibodies heated in this work behaved similarly, and the apparent differences

in the heat stability of the antisera resulted from differences in the competitive action of the "mixed complexes."

Why "mixed complexes" fail to flocculate the antigen, although they combine with it, is unknown. The amount and the properties of the material forming complexes with antibody particles during heating, however, probably determine this. If, like albumin, it readily forms stable suspensions, union with antigen is less likely to give an insoluble product than if, like heated globulin, it forms unstable suspensions. Similarly, the formation of complexes composed largely of serologically unspecific material can change the behaviour of proteins which function as antigens (Bawden and Kleczkowski, 1941).

The formation of serologically active antibody-complexes occurs only over a limited range of heating, further heating leading to a loss of all serological activity. This applies equally to both "O" and "H" type of antigens. This change is reflected in the drop of flocculating titre of heated euglobulin solutions and in the disappearance of inhibition in solutions of "mixed complexes." This stage probably corresponds with a further stage of protein denaturation, in which the antibody particles are so altered that they no longer contain specific groups capable of combining with antigen.

SUMMARY.

Experiments on the effect of heat on rabbit antisera to the following antigens have been made: human serum albumin and globulin, a strain of pea nodule bacteria (*Rhizobium leguminosarum*); and purified preparations of the following plant viruses—tobacco mosaic, potato "X," tomato bushy stunt and tobacco necrosis.

Antisera to the rod-shaped viruses (tobacco mosaic and potato "X") behave like those to flagellar type antigens, whereas antisera to the other antigens named behave like those to somatic type antigens, much more heating being needed to destroy the flocculating power of the former. However, euglobulin fractions (containing antibodies) of all the antisera behave similarly, and they require more heat to destroy their flocculating power than do the original antisera.

Flocculating antibodies undergo at least two changes during heating. Complexes composed of antibody particles and of particles of other unspecific proteins present in the solution are first formed. Antibodies changed in this way can still combine specifically with antigens, but the result of this combination depends on the quantity and quality of unspecific proteins present in the solution during heating. Complexes formed when antibodies are heated in the presence of euglobulin fraction of the antiserum flocculate their antigens. Complexes formed when antibodies are heated in the presence of other serum fractions, notably albumin, cannot flocculate their antigens, although they combine with them; this combination interferes with the flocculating action of antibodies that are unchanged. The degree of this interference depends on the type of antigen, being large with antigens of type "O" and small with those of type "H." This fact, and not a difference in heat stability, explains the differences in the behaviour of heated antisera to the two types of antigen.

The second change of antibodies during heating corresponds with a further stage of denaturation and is shown by the loss of ability to combine with antigen.

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SOME PROPERTIES OF COMPLEXES FORMED WHEN ANTIGENS ARE HEATED IN THE PRESENCE OF SEROLOGICALLY UNSPECIFIC PROTEINS.

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KLECZKOWSKI (1941*b, c*) has presented evidence suggesting that rabbit antibodies can combine with other proteins while undergoing denaturation by heat. The serological behaviour of the complexes so formed depends on the protein with which the antibody has combined and on the type of antigen. Antibody-euglobulin complexes behave much like unchanged antibody, whereas antibody-albumin complexes can combine with, but not flocculate their antigens. Flagellar type antigens are much more readily flocculated by mixtures containing both unchanged antibody and antibody-albumin complexes than somatic type antigens. Because of this and not because of a greater resistance to heat, as previously believed, antisera to flagellar type antigens can be heated more than antisera to somatic type antigens without losing their ability to cause flocculation.

This paper shows that the serological behaviour of antigens after heating depends in the same way on what other proteins are present during heating and on whether the antigen is of the flagellar or somatic type. The antigens used were tomato bushy stunt and tobacco mosaic viruses and human serum

albumin and globulin. Tobacco mosaic virus is an antigen of the flagellar type giving a loose nebulous precipitate, and the others are of the somatic type, giving dense granular precipitates. The antigens and antisera were prepared and tested by the methods previously described.

The effects of heating tomato bushy stunt virus in the presence and absence of albumin.

When 0.05 per cent. solutions of tomato bushy stunt virus in saline at pH 7 are heated for 10 minutes at 80° C. they become opalescent, and a small sediment can be deposited by centrifuging at 8000 r.p.m., leaving an opalescent supernatant fluid. At 83° C. the fluids become increasingly opalescent, and although no floccules separate on standing, most of the material sediments on centrifuging to leave a water-clear supernatant fluid. Heating at 86° C. or higher causes the protein to settle out spontaneously. When the virus solution is heated in the presence of 0.5 per cent. rabbit-serum albumin it behaves

TABLE I.—*Effect of Heating Bushy Stunt Virus Solutions in the Presence and Absence of Rabbit Serum Albumin.*

0.05 per cent. solutions of bushy stunt virus in physiological saline and in 0.5 per cent. rabbit serum albumin solutions were heated for 10 minutes at different temperatures at pH 7.0.

1 ml. of the bushy stunt virus preparations diluted 1/10 in saline were added to a series of tubes each containing 1 ml. of varying dilutions of a bushy stunt virus antiserum.

+ indicates precipitation and - no precipitation.

After 3 hours 0.1 ml. of 0.05 per cent. solution of control bushy stunt virus was added to the tubes containing no precipitate.

i indicates that there was still no precipitation (inhibition) and o indicates the separation of precipitate (no inhibition).

Temperature. of heating.	Solvent.	Dilution of the antiserum.					
		1/10.	1/20.	1/40.	1/80.	1/160.	1/320.
Unheated	Saline	+	+	+	+	—	—
80° C.	Saline	+	+	+	+	+	—
„	Albumin solution	—	—	—	—	—	—
		o	i	i	i		
83° C.	Saline	+	+	+	+	+	+
„	Albumin solution	—	—	—	—	—	—
		i	i	i	i		
90° C.	„	—	—	—	—	—	—
		i	i	i	i		
95° C.	„	—	—	—	—	—	—
		o	o	o	o		

Solutions of bushy stunt virus and of rabbit albumin in saline mixed after being heated separately.

80° C.	+	+	+	+	+	—
83° C.	+	+	+	+	+	+

quite differently. Solutions heated at 80° and 83° do not become opalescent, and no precipitate separates even after heating at 95° C., although with this treatment the fluid becomes highly viscous and opalescent.

The infectivity of the virus solutions is destroyed by 10 minutes heating at 80° C., both in the presence and absence of albumin, but the ability to combine with virus-antibody is retained. The serological behaviour of the heated solutions is shown in Table I. The stable opalescent suspensions produced by heating the virus in saline at 80° C. and 83° C. are flocculated specifically by virus-antiserum. Floccules form sooner and the suspensions are flocculated by higher dilutions of antiserum than solutions with the same concentration of unheated virus. By contrast, no flocculation occurs when preparations of bushy stunt virus heated at between 80° C. and 90° C. in the presence of albumin are added to virus-antiserum. Although there is no flocculation, the heated virus-albumin mixture does combine with the virus-antibody, for it inhibits flocculation when unheated virus is added subsequently. In other words, heating the virus in the presence of albumin has produced something behaving like a non-precipitating hapten, able to combine with antibody and to prevent it from flocculating unchanged virus.

Heating for 10 minutes at 95° C. in the presence of albumin destroys the ability to combine with virus-antibody, for when such preparations are tested against virus antiserum they fail to flocculate or to inhibit the flocculation of unheated virus added subsequently. Inhibition only occurs when the virus and albumin are heated together at temperatures between 80 and 90° C. If the two are heated separately and then mixed, the mixtures flocculate with antiserum in the same way as heated virus in the absence of albumin. Thus the non-precipitating hapten is formed only when the virus and albumin are heated together over a limited range of temperature. For the inhibition to be complete there must also be considerably more albumin in the heated fluids than virus. In Table I where inhibition is complete, the ratio of albumin to virus was 10 to 1, but with a ratio of 4 to 1 flocculation with antiserum still occurs, although much more slowly than with unheated virus.

Table II shows that the material rendered sedimentable by heating solutions of bushy stunt virus in saline for 10 minutes at 80° and 83° C. is still flocculated by virus-antiserum. This was shown by taking up the sedimented material in saline, in which it formed stable suspensions, and testing against virus-antiserum, which flocculated it specifically. The denatured protein that separates spontaneously from virus solutions heated at 86° C. does not resuspend to give stable suspensions, but if sufficiently diluted it remains stable long enough to demonstrate that it is flocculated specifically by virus-antiserum. The material separating at 90° and higher is too unstable for such tests, for it flocculates rapidly in the presence of normal rabbit serum.

The ability of the precipitates formed by heating bushy stunt virus at different temperatures to combine with antibody was tested in another way. Suspensions of the heated virus were mixed with antiserum at various dilutions, incubated for 3 hours at 50° C., and the precipitates removed by centrifugation. The supernatant fluids were then tested for their antibody content by the addition of unheated virus. The unabsorbed serum reacted at a dilution of

TABLE II.—*Effect of Heating Bushy Stunt Virus Solution in Physiological Saline.*

0.05 per cent. solutions of bushy stunt virus in saline at pH 7.0 were heated for 10 minutes at different temperatures. The heated suspensions (if stable), the supernatant fluids obtained after centrifuging at 8000 r.p.m. and the precipitates resuspended in the original volume of saline were used for serological tests.

First test.

1 ml. of each preparation of bushy stunt virus at a dilution of 1/10 in saline was added to a series of tubes each containing 1 ml. of varying dilution of antiserum.

+ indicates flocculation and – no flocculation.

Second test.

After 3 hours the contents of the tubes were centrifuged and 0.1 ml. of 0.05 per cent. virus solution was added to the supernatant fluids.

+ indicates flocculation (presence of unabsorbed antibodies) and – indicates no flocculation.

Temperature of heating.	Preparation of bushy stunt virus.	Dilutions of antiserum.									
		First test.						Second test.			
		1/10.	1/20.	1/40.	1/80.	1/160.	1/320.	1/10.	1/20.	1/40.	1/80.
Unheated	Solution	+	+	+	+	—	—	+	—	—	—
80° C.	Orig.	+	+	+	+	+	—				
	suspension										
„	Supernatant	+	+	+	+	+	—				
„	Precipitate	—	—	—	+	+	+				
83° C.	Orig.	+	+	+	+	+	+	+	+	—	—
	suspension										
„	Supernatant	—	—	—	—	—	—				
„	Precipitate	+	+	+	+	+	+	+	+	—	—
86° C.	Supernatant	—	—	—	—	—	—				
„	Precipitate ½ hr.	—	—	+	+	+	+				
	1 hr.	+	+	+	+	+	+	+	+	—	—
90° C.	„ ½ hr.	+	+	+	+	+	+	+	+	+	—
95° C.	„ ½ hr.	+	+	+	+	+	+	+	+	+	+

* Unspecific flocculation due to instability of the suspension and formed also in the controls with normal rabbit-serum.

1/80 and after absorption with unheated virus it reacted at 1/10. The precipitation end-point after absorption with material precipitated at 83°, 86°, 90°, and 95° was 1/20, 1/20, 1/40, and 1/80 respectively. Thus the virus precipitated by heating at 83° and 86° combined with approximately half as much antibody as the same amount of unheated virus and that heated at 90° C. with a quarter as much, whereas virus heated at 95° C. combined with no antibody. The amount of heating required to denature and precipitate bushy stunt virus, therefore, is less than that required to destroy its ability to combine

with antibody, and this is apparently the same whether the virus is heated alone or in the presence of albumin.

Four changes can be detected in bushy stunt virus preparations heated for 10 minutes at different temperatures. Below 80° C. there is loss of infectivity, with no change in the serological reactions, and at 95° C. and higher the virus loses its ability to combine with its antibody. The serological behaviour after heating at temperatures between 80° C. and 95° C. depends on whether the virus is heated alone or with albumin. If heated in saline around 83° C. the solutions become opalescent and are more easily flocculated by antiserum, whereas at 86° C. or higher the virus becomes insoluble, though still able to combine with antibody. When heated with albumin in this range the virus remains in suspension and able to combine with antibody, but is not flocculated by the combination.

The effects of heating tobacco mosaic virus in the presence and absence of albumin.

When tobacco mosaic virus antiserum is heated at temperatures around 80° C. it continues to flocculate its antigen whereas bushy stunt virus antiserum does not (Kleczkowski, 1941c). Similarly, the serological behaviour of the two viruses differs when they are heated in the presence of albumin, for although tobacco mosaic virus interacts with albumin, the product of heating does not behave like a non-precipitating hapten. The appearance of heated preparations of tobacco mosaic virus depends on the pH and on whether albumin is present. 0.05 per cent. solutions of virus in *M*/15 phosphate buffer at pH 6 become slightly opalescent after 10 minutes at 80° C. and still show the phenomenon of anisotropy of flow strongly, whereas at pH 7 much of the material separates as a precipitate and anisotropy of flow disappears. After 10 minutes at 90° C. precipitates separate at pH 6 as well as pH 7, and anisotropy of flow is lost. When 0.05 per cent. virus solutions are heated in the presence of 0.6 per cent. rabbit serum albumin at pH 7, no precipitates separate after 10 minutes heating at 80° C. or 90° C. After heating at 80° C. there is some increased opalescence and the anisotropy of flow is reduced, though not destroyed, and at 90° C. the fluids become extremely opalescent and viscous and no anisotropy of flow can be detected.

Table III shows the effects on the infectivity and serological activity of heating tobacco mosaic virus for 10 minutes at 80° and 90° C. under different conditions. The conditions under which the fluids are heated at 80° C. greatly affect the activity of the virus. At pH 7 both infectivity and serological activity are reduced to about one-twentieth of the control, and the residual activity is in the remaining soluble material. At pH 6 there is no loss of serological activity and a small drop in infectivity. The presence of albumin in the virus solutions heated at pH 7 protects them almost completely from inactivation. The mere addition of albumin, either heated or unheated, to the virus solutions causes a reduction in infectivity, the reduction being much less apparent after diluting the mixtures, but there is little further loss when the mixtures are heated for 10 minutes at 80° C., and only a small drop in serological activity. Heating for 10 minutes at 90° C. has destroyed both infectivity and

TABLE III.—*Effect of Heating Tobacco Mosaic Virus Solutions in Different Conditions.*

0.05 per cent. virus solutions were heated for 10 minutes in saline at pH 7.0, in phosphate buffer at pH 6.0 and in 0.6 per cent. rabbit serum albumin solution at pH 7.0.

Prep. no.	Temperature of heating.	pH.	Diluent.	Serological titre of the preparation.	Average number of lesions per leaf at	
					1/1.	1/20.
1 .	Unheated	7.0 .	Saline	1/160 .	115	53
2 .	80° C.	6.0 .	Phosph. buffer	1/160 .	103	37
3 .	"	7.0 .	Saline	" .	27	10
4 .	Supernatant from (3)			1/8 .	35	11
5 .	Resuspended precipitate from (3)			" .	0.5	0
6 .	80° C.	7.0 .	Albumin solution	1/120 .	21	28
7 .	Unheated	7.0 .	"	1/160 .	32	34
			heated at 80° C.			
8 .	"	7.0 .	Unheated albumin solution	1/160 .	26	29
9 .	90° C.	6.0 .	Phosph. buffer	" .	0	0
10 .	Supernatant from (9)			No pptt. at 1/2.		
11 .	90° C.	7.0 .	Saline	" .	0	0
12 .	Supernatant from (11)			No pptt. at 1/2.		
13 .	90° C.	7.0 .	Albumin solution	" .	0	0

the serological reactions. The ability to combine with antibody was destroyed both by heating alone and in the presence of albumin, for incubating antiserum with the material precipitated by heat did not reduce its antibody content, and incubation with the heated virus-albumin mixture did not prevent it from flocculating active virus added subsequently.

The disruption by heat and loss of all specific properties seems to occur more suddenly with tobacco mosaic virus than with bushy stunt virus, and fewer successive changes can be detected on heating. At pH 6 there is a suggestion that infectivity is being lost before denaturation or loss of serological activity occurs, and similar separations of these two activities have been described earlier (Lauffer and Price, 1940; Bawden and Pirie, 1940), but the separation is slight compared with bushy stunt virus. The protein that separates from denatured tobacco mosaic virus preparations is free from nucleic acid, whereas that from bushy stunt virus contains nucleic acid, suggesting that the nucleic acid-protein linkage is more stable in bushy stunt than in tobacco mosaic virus. This may explain why the precipitates formed on heating bushy stunt virus still combine with virus-antibody whereas those from tobacco mosaic virus do not, for it may be that the nucleic acid-protein link is necessary for serological specificity. Alternatively, it may be that the breaking of such a linkage is followed immediately by the loss of all structural specificity, whereas in bushy stunt virus other, structurally less important linkages are more sensitive to heating, and the breaking of these one-by-one is responsible for each stage that can be detected in the loss of specific activities on heating.

The effect of albumin in protecting tobacco mosaic virus against heat-inactivation suggests that denaturation may be in part a reversible reaction. In the absence of albumin the end-product is insoluble and is removed from the reaction, which can therefore rapidly proceed to completion. But in the presence of albumin there is no precipitation and this may slow down the reaction.

The effects of heat on the serological reactions of human serum proteins.

Previous work on the influence of heat on the antigenic specificity of animal sera has given conflicting results, for some workers have stated that heating destroys all the original specificity whereas others have stated that it merely causes modifications (e.g. Obermayer and Pick, 1904; Furth 1925). Spiegel-Adolf (1926) showed that heated serum could combine with its antibody without being precipitated and that this combination prevented precipitation when unheated serum was added later. Antibody made in one animal by the injection of another animal's serum is often predominantly to globulin (Kleczkowski, 1938), and albumin then represents a serologically unspecific protein. The conflicting results may therefore arise from the fact that the antisera used by different workers varied in their content of antibody to globulin and albumin, for our results show that the serological behaviour of heated antigenic proteins is greatly modified by the presence of serologically unspecific proteins during heating.

Table IV shows the results of precipitin tests with 0.05 per cent. solutions of whole globulin from human serum after ten minutes' heating at 80° C. in saline and in 0.5 per cent. solutions of rabbit serum albumin and human albumin. After heating in saline the globulin is still precipitated by its anti-serum, but after heating in the presence of either of the albumins there is no precipitation and the presence of the heated proteins prevents the precipitation of unheated globulin added later. The heated mixture of globulin and human albumin, however, was still precipitated by albumin antiserum.

When human albumin is heated at 80° C. in the presence of rabbit serum albumin its serological behaviour is altered so that it combines with antibody but is not precipitated by it (Table VI). As with globulin-albumin mixtures the ability to inhibit the precipitation of unheated antigen is developed only when the serologically specific and unspecific proteins are heated together and not when they are heated separately and then mixed.

When heated at 80° C. in the presence of serologically unspecific albumin, human globulin and albumin differ in their serological behaviour from tobacco mosaic virus and resemble bushy stunt virus, i.e. all the somatic type antigens tested behave in the same way. Their serologically active groups, however, are more resistant to heat than those of bushy stunt virus, for human albumin and globulin continue to precipitate with their antisera after heating separately for 5 minutes at 100° C. (Tables V and VII). Similarly, when heated at this temperature in the presence of twice their concentration of serologically unspecific protein, both human albumin and globulin continue to combine with their antibodies and to behave like non-precipitating haptens.

TABLE IV.—*Effect of Heating for 10 Minutes at 80° C. Human Serum Globulin Solution alone and in the Presence of Human or Rabbit Serum Albumin.*

0.05 per cent. solutions of human rabbit serum globulin were heated in saline and in 0.5 per cent. human or rabbit serum albumin solutions.

1 ml. of an antiserum to human serum globulin diluted 1/20 was added to a series of tubes each containing 1 ml. of varying dilutions of human globulin.

+ indicates precipitation and — no precipitation.

After 3 hours 0.1 ml. of 0.06 per cent. solution of human serum globulin was added to the tubes in which there was no precipitation.

i indicates that there was still no precipitation (inhibition) and o indicates the separation of precipitate (no inhibition).

Preparation of human globulin.	Amount of human globulin (in mg.).				
	0.25	0.12.	0.06.	0.03.	0.015.
Unheated solution in saline . . .	+	+	+	+	+
Heated solution in saline . . .	+	+	+	+	—
Heated solution with human albumin*	—	—	—	—	—
	i	i	o	o	o
Heated solution with rabbit albumin	—	—	—	—	—
	i	i	o	o	o
Solution in saline mixed with human albumin solution after heating separately	+	+	+	+	—
					o
Solution in saline mixed with rabbit albumin solution after heating separately	+	+	+	+	—
					o

* This preparation precipitated with antiserum to human serum albumin.

TABLE V.—*Effect of Heating for 5 Minutes at 100° C. Human Serum Globulin Solutions alone and in the Presence of Human Albumin.*

0.1 per cent. solutions of human serum globulin were heated in saline and in 0.2 per cent. human serum albumin solution.

The antiserum to human serum globulin was used at a dilution of 1/40, otherwise the methods of testing were as described for Table IV.

Preparation of human globulin.	Amount of human globulin (in mg.).					
	0.5.	0.25.	0.12.	0.06.	0.03.	0.015.
Unheated solution	+	+	+	+	+	+
Heated solution in saline	+	+	+	+	+	—
Heated solution with human albumin	—	—	—	—	—	—
	i	i	o	o	o	o
Solution in saline mixed with human albumin solution heated separately	+	+	+	+	+	—

TABLE VI.—*Effect of Heating for 10 Minutes at 80° C. Human Serum Albumin Solutions alone and in the Presence of Rabbit Serum Albumin.*

0.05 per cent. solutions of human serum albumin were heated in saline and in 0.5 per cent. rabbit serum albumin solution.

1 ml. of an antiserum to human serum albumin diluted 1/40 was added to a series of tubes each containing 1 ml. of varying dilutions of human serum albumin. After 3 hours 0.1 ml. of 0.06 per cent. solution of human serum albumin was added to the tubes where there was no precipitation.

Preparation of human albumina.	Amount of human albumin (in mg.).			
	0.06.	0.03.	0.015.	0.0075.
Unheated solution	+	+	+	+
Heated solution in saline	+	+	+	+
„ „ with rabbit albumin	—	—	—	—
	i	i	i	i
Solution in saline mixed with rabbit albumin solution after heating separately	+	+	+	+
Heated 0.5 per cent. human albumin solution in saline	+	+	+	+

TABLE VII.—*Effect of Heating for 5 Minutes at 100° C. Human Serum Albumin Solutions alone and in the Presence of Rabbit Serum Albumin.*

0.1 per cent. solutions of human serum albumin were heated in saline and in 0.2 per cent. rabbit serum albumin solution.

Methods were as in Table VI.

Preparation of human albumin.	Amount of human albumin (in mg.).			
	0.06.	0.03.	0.015.	0.0075.
Unheated solution	+	+	+	+
Heated solution in saline	+	+	+	+
„ „ with rabbit albumin	—	—	—	—
	i	i	o	o
Solution in saline mixed with rabbit albumin solution after heating separately	+	+	+	+

The precipitation of viruses heated with albumin by human albumin antiserum.

The most reasonable explanation of the change in the serological behaviour of somatic type antigens after heating in the presence and absence of albumin is that the antigens combine with other proteins while undergoing heat denaturation to produce complexes, which remain soluble after uniting with antibody. Positive evidence for such a combination between bushy stunt virus and human albumin was supplied when it was found that the virus, heated in the presence of human albumin, could be precipitated by human albumin antiserum. 0.05 per

cent. solutions of bushy stunt virus in saline and in 0.6 per cent. solution of human albumin were heated for 10 minutes at 80° C. The virus heated in saline was precipitable by virus antiserum, whereas that heated with albumin was not, and inhibited the precipitation of unheated virus, although it precipitated with antiserum to human albumin. Antiserum to human albumin was allowed to react at the constant serum optimal proportions with the virus and albumin heated together and with a mixture of the same proportions of the two heated separately. After two hours at 37° C. and 48 hours at 1° C., the fluids were centrifuged and the supernatant fluids tested against antiserum to bushy stunt virus. The virus and albumin heated together now neither precipitated with virus antiserum nor inhibited the precipitation of unheated virus added later, whereas the mixture of virus and albumin heated separately still precipitated with virus antiserum to the same extent as before it was absorbed with human albumin antiserum.

As the precipitation and the inactivation of tobacco mosaic virus by heat at pH 7 is retarded by the presence of albumin, it is obvious that there is an interaction between the two. An experiment similar to that described with bushy stunt virus showed that tobacco mosaic virus also combines with albumin when the two are heated together. 0.05 per cent. solutions of tobacco mosaic virus were heated for 10 minutes at 80° C. in the presence of 0.6 per cent. human albumin, and samples were allowed to react fully with antiserum to human albumin. Before reacting with this serum the heated mixture of virus and albumin precipitated strongly with virus antiserum, but after absorption with human albumin antiserum it did not. A mixture of unheated virus and heated albumin, however, precipitated with virus antiserum equally before and after absorption with antiserum to human albumin. Thus with both viruses it is only when they have been heated in the presence of the albumin that they can be removed by absorption with human albumin antiserum.

DISCUSSION.

Evidence that serum proteins can combine when heated together has recently come from a number of sources. Kleczkowski (1941*b*) has shown that a new component readily sedimentable appears in heated rabbit serum, and Van der Scheer *et al.* (1941) and Krejci *et al.* (1941) have shown by electrophoretic studies that new components appear in horse serum, both normal and anti-pneumococcal, after heating. The results described in this paper show that other proteins, as different as plant viruses and serum proteins, can also combine when undergoing heat denaturation together. The complexes formed may have different physical properties from either of the two components heated separately. For example, when euglobulin is heated with albumin there is no spontaneous precipitate of denatured protein, but a material is produced which is deposited as a gel on moderate centrifugation and can be dispersed in water to give a stable suspension. Similarly, when bushy stunt and tobacco mosaic viruses are heated with albumin they give no such precipitates as when heated alone, but the complexes they form with albumin must be smaller than that formed by globulin, for nothing can be sedimented from the heated mixtures of viruses and albumin by centrifuging at 8000 r.p.m.

It has previously been suggested (Steinhardt, 1938 ; Bawden and Pirie, 1940) that the efficiency of denaturing agents depends on their ability to break cross linkages between peptide chains in proteins, and that the probability of the linkages returning to their original pattern on the removal of the agent is lessened by the increase in the number of such linkages broken. If denaturation by heat is also assumed to result from breakage of cross linkages, our results can be explained on the basis that the linkages are reformed purely at random, and that when more than one protein is being heated cross linkages are as likely to reform between chains in different proteins as between chains in the same protein. If this is so, then it is likely that complexes will only be formed between proteins in which similar cross linkages are being broken at the same time, and that those that denature at widely different temperatures will not combine in the same way. All the proteins used in this work undergo demonstrable changes when heated alone for a few minutes around 80° C., which is the amount of heating required to produce complexes when they were heated together.

Although it is possible that combination can occur between other proteins possessing similar heat stabilities, the effects on the serological reactions of the proteins might be expected to differ from those described here. All the serological changes were produced by heating antigens in the presence of serologically unspecific albumin and tended to make the antigen less precipitable by its antibody. Heated albumin is very stable and combination between antigens and other, less stable proteins might be expected to give different results.

The fact that tobacco mosaic virus after heating with albumin still flocculates with its antiserum, whereas somatic types antigen do not, cannot be attributed to a failure of this virus to unite with albumin, for that combination does occur is shown by the reduction in the rate of inactivation by heat, by the fact that no spontaneous precipitate of denatured protein separates, and by the precipitation of virus heated with albumin by albumin antiserum. Tobacco mosaic virus is more readily flocculated by its antibody than antigens of the somatic type ; floccules separate quicker, and they are produced with less antibody and over a much wider range of antigen/antibody ratios. (Kleczkowski, 1941*a*). This greater insolubility of the tobacco mosaic virus-antibody combination may explain the differences in the serological behaviour of flagellar and somatic type antigens after heating with albumin. A similar difference is found when antisera to the two kinds of antigen are heated, for after the same amount of heating those to somatic type antigens do not cause flocculation but those to flagellar type antigens do. When the ratio of albumin to antibody is increased by about three times that in serum, however, heating causes antibodies to tobacco mosaic virus to behave like those to somatic type antigens and to inhibit flocculation. Thus it seems that combination of either somatic type antigens or their antibodies with relatively small amounts of soluble, serologically unspecific protein increases the solubility of the complex formed by the union of antigen and antibody sufficiently to prevent flocculation, whereas a complex containing much more unspecific protein is necessary to keep the combination of flagellar type antigen and antibody in solution.

SUMMARY.

When tomato bushy stunt virus, human serum globulin and albumin are heated at temperatures around 80° C. in the absence of serologically unspecific proteins, they are still able to precipitate with their specific antisera. If heated in the presence of serologically unspecific serum albumin, however, they produce complexes behaving like non-precipitating haptens. Human globulin, and albumin behave in this way after being heated for 5 minutes at 100° C., but bushy stunt virus does not combine with antibody after heating for 10 minutes at 95° C.

The presence of albumin in solutions of tobacco mosaic virus heated at pH 7.0 reduces the rate of inactivation, but this flagellar type antigen still flocculates with its antiserum. This virus loses its ability to combine with antibody after 10 minutes at 90° C.

Heating either virus in the presence of albumin prevents the separation of precipitates of denatured protein, and that combination between the viruses and albumin occurs is shown by the fact that the viruses can then be removed by precipitation with antiserum to the albumin.

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THE FORMATION OF PROTEIN COMPLEXES IN HEATED SOLUTIONS OF RABBIT SERUM PROTEINS.

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THE results of heating euglobulin fractions of rabbit antisera showed that the presence of other serum fractions during heating leads to the formation of changed antibodies which can unite with their antigens but not flocculate them (Kleczkowski, 1941). Heating euglobulin fractions of antisera in the absence of other serum fractions does not produce such changes in the serological behaviour of antibodies. In order to understand the possible interaction between antibodies and other serum proteins, the behaviour of normal rabbit serum proteins on heating has been studied, and this paper describes the differences obtained when rabbit serum euglobulin and albumin solutions are heated separately and together.

METHODS AND MATERIAL.

Preparation of protein fractions.—Normal rabbit serum was 1/3 saturated with ammonium sulphate, the precipitate filtered off and washed with 1/3 saturated ammonium sulphate solution. The precipitate was dissolved in water and dialysed, and the insoluble euglobulin was filtered off. The filtrate was used as soluble euglobulin.

The filtrate from serum 1/3 saturated with ammonium sulphate was 1/2 saturated and the precipitate of pseudoglobulin filtered off. The filtrate was then fully saturated with ammonium sulphate, the precipitate redissolved in water and dialysed. The solution was used as albumin fraction.

The nitrogen content of the dialysed solutions was determined by micro-Kjeldahl, and this was translated into protein content by multiplying by 6.25.

The protein solutions were heated in 0.9 per cent. NaCl solution at pH 6.8. They were heated in thin-walled tubes in a water-bath, and the time of heating was taken from the moment when the fluids reached the temperature of the bath, which was usually about one minute after immersion.

To determine the amount of protein precipitated from heated protein solutions by the addition of ammonium sulphate, the fluid was allowed to stand undisturbed for at least two days, when the precipitate had settled. Then to a measured volume of the supernatant fluid trichloroacetic acid was added to make a concentration of 3.3 per cent., and the fluid was heated for a few minutes at 100° C. The resulting precipitate was centrifuged down and washed repeatedly

with 3.3 per cent. trichloroacetic acid until no test for SO_4^{2-} was obtained with BaCl_2 . The amount of protein was then determined by micro-Kjeldahl.

RESULTS.

Table I shows that when solutions of euglobulin and albumin were heated for 10 minutes at 80°C . in identical conditions a precipitate separated from the first (Exps. Nos. 2 and 5) but not from the second (Exp. No. 1), and when mixtures of the two were heated their appearance depended on the proportions in which they were mixed (Exps. Nos. 3 and 4). All the heated solutions, however, gave a strong nitroprusside test (made as described by Harris, 1923), showing that they all had undergone changes producing active disulphide groups.

TABLE I.—*The Behaviour of Rabbit Serum Albumin and Euglobulin in Solutions Heated Separately and Together.*

The protein solutions were heated for 10 minutes at 80°C . in 0.9 per cent. NaCl at pH 6.8.

No. of experiment.	Amount (mg.) of protein in 10 ml. of solution.		Appearance of the fluid after heating.	Amount (mg.) of protein in the precipitate from 10 ml. of the solution.
	Albumin.	Euglobulin.		
1	50.0	0	Very slight opalescence	0
2	0	50.0	Precipitation	39.0
3	25.0	25.0	"	34.0
4	37.5	12.5	Opalescence*	0
5	0	12.5	Precipitation	8.6

* 5.0 mg. of a yellow gelatinous sediment was obtained by centrifugation at 8000 r.p.m.

When the ratio albumin/euglobulin in the mixture was sufficiently in favour of albumin, for instance when it was 3/1 (Exp. No. 4), no precipitate separated on standing. However by centrifugation at 8000 r.p.m. a yellow gelatinous sediment was obtained. This could be taken up in water to form a stable, slightly opalescent suspension. The mixture of equal parts of the two fractions (Exp. No. 3) gave a precipitate larger than the total amount of either constituent. Therefore, although albumin heated alone gave no precipitate, it is evident that protein separating as a precipitate from the heated mixture originated from both constituents. This suggests that when the two fractions are heated together a complex composed of heat-changed protein originating from both fractions is formed and its stability in suspension depends on the ratio of the two constituents. The experiments described below, in which precipitability by ammonium sulphate was used as an indication of changes produced by heating, show that this complex is produced *during* heating, for albumin and globulin solutions mixed after being heated separately do not influence each other's properties.

For estimating the precipitability by ammonium sulphate, 4 ml. samples of 0.5 per cent. protein solutions were heated, diluted to 10 ml. with water, and saturated ammonium sulphate solution was added to the required concentration. The amounts of precipitate are shown in Table II.

TABLE 11.—*Precipitability of Euglobulin, Albumin, and of a Mixture of Equal Parts of the Two, after Heating for Varying Lengths of Time at 75° C.*

The proteins were heated as 0.5 per cent. solutions in 0.9 per cent. NaCl at pH 6.8.

Protein.	Precipitate formed.	The amounts of precipitate (as percentage of total protein) after heating.				
		Minutes heating.				
		30.	40.	120.	160.	320.
Euglobulin.	Spontaneously	0	22	55	55	55
Albumin-euglobulin		0	0	0	0	0
Albumin		0	0	0	0	0
Euglobulin.	With 20 per cent. saturated (NH ₄) ₂ SO ₄	75	79	82	82	84
Albumin-euglobulin		0	0	0	0	43
Albumin		0	0	0	0	0
Albumin-euglobulin	With 28 per cent. saturated (NH ₄) ₂ SO ₄	56	65	76	76	80
Albumin		0	0	0	40	65
Albumin	With 42 per cent. saturated (NH ₄) ₂ SO ₄	64	65	67	67	69

When euglobulin solutions were heated at 75° C. for more than 30 minutes a precipitate separated spontaneously. No precipitate separated from albumin and albumin-euglobulin mixtures of equal parts heated for as long as 320 minutes, but the fluids became increasingly opalescent with increased heating and all heated solutions gave a positive nitroprusside test.

The precipitability of heated albumin-euglobulin mixture differed considerably from that of either albumin or euglobulin heated separately. After a given period of heating the degree of precipitability of the mixture was intermediate between those of euglobulin and albumin. After 120 minutes' heating at 75° C. 20 per cent. saturated ammonium sulphate precipitated only euglobulin, and 28 per cent. saturation precipitated the mixture, but not albumin heated alone. The amount of protein precipitated from the mixture after 120 minutes' heating by 28 per cent. saturated ammonium sulphate was 76 per cent. of the total amount of protein. It is obvious, therefore, that the precipitate produced in the heated mixture must have been composed of material originating from both fractions, although this concentration of ammonium sulphate has no effect on solutions of albumin heated alone for 120 minutes. Thus the product of heating the mixture was formed at the expense of both constituents, and was more easily precipitable by (NH₄)₂SO₄ than albumin heated alone.

On the other hand, after 160 minutes' heating the albumin-euglobulin mixture is not precipitable by 20 per cent. saturated ammonium sulphate, whereas 55 per cent. of euglobulin heated alone precipitated spontaneously, and the amount of precipitate obtained by 20 per cent. saturated ammonium sulphate (together with the precipitate formed spontaneously) was 82 per cent. of total protein. Therefore, the product of heating the mixture forms a more stable suspension than euglobulin heated alone.

If albumin and euglobulin are heated separately and then mixed, they are precipitated by ammonium sulphate just as they are alone. This is illustrated by Table III. Table IIIA shows the results of heating for 20 minutes at 75° C. 28 per cent. saturated ammonium sulphate did not precipitate albumin heated alone (Exp. No. 1), but it precipitated heated albumin-euglobulin mixture (Exp. No. 2). 20 per cent. saturation did not precipitate the mixture (Exp. No. 3), but it precipitated euglobulin heated alone (Exp. No. 4), and the addition of albumin previously heated separately did not prevent the precipitation nor influence the amount of precipitate (compare Exps. Nos. 5 and 4).

TABLE III.—*Precipitation by Ammonium Sulphate of Heated Albumin, Euglobulin and a Mixture of the Two.*

Exp. No.	Volume (ml.) of heated 0.5 per cent. protein solution taken.			Volume of H ₂ O added (ml.).	Volume of satur. (NH ₄) ₂ SO ₄ added (ml.).	Per cent. of saturation with (NH ₄) ₂ SO ₄ .	Appearance of the fluid after addition of (NH ₄) ₂ SO ₄ .	Amount of protein in the precipitate (mg.).
	Euglobulin.	Albumin.	Albumin-euglobulin mixture (1/1).					

The proteins were heated as 0.5 per cent. solutions in 0.9 per cent. NaCl at pH 6.8.

A. *Heating for 20 minutes at 75° C.*

1	0	4	0	6	4.0	28	Clear	0
2	0	0	4	6	4.0	28	Precipitate	10.2
3	0	0	4	6	2.5	20	Slight opalescence	0
4	2	0	0	8	2.5	20	Precipitate	7.0
5	2	2	0	6	2.5	20	..	6.8

B. *Heating for 15 minutes at 70° C.*

6	0	4	0	6	4.0	28	Clear	0
7	0	0	4	6	4.0	28	Slight opalescence	0
8	2	0	0	8	4.0	28	Precipitate	3.4
9	2	2	0	6	4.0	28	..	3.6

Table IIIB shows the results of heating for 15 minutes at 70° C. 28 per cent. saturated ammonium sulphate did not precipitate albumin heated alone or the heated albumin-euglobulin mixture (Exps. Nos. 6 and 7), but it precipitated euglobulin heated alone, and the amount of precipitate was almost the same whether heated albumin solution was added or not (compare Exps. Nos. 8 and 9).

The results in Table IIIA and B show that only when the two proteins are heated together is their precipitability affected. The separation of floccules, or the production of a material forming stable suspensions which can be sedimented as a gel by centrifugation at moderate speeds, shows that large aggregates are produced during heating. The formation of a product composed of protein originating from both fractions, with properties different

from those of either constituent heated separately, indicates that combination between particles of both fractions occurs during heating. The appearance of active disulphide groups indicates at least an early stage of denaturation, so that the large colloidal complexes formed during heating are formed of at least partly denatured protein particles. The combination of protein particles to form these complexes thus coincides with a change of their original structure.

Recently Van der Scheer, Wyckoff and Clarke (1941) arrived at similar conclusions from their electrophoretic studies of heated horse serum, which showed that a new colloidal aggregation product resulting from denaturation of components of the serum is formed at the expense of both the albumin and the globulin.

SUMMARY.

The effects of heating normal rabbit serum albumin and euglobulin fractions separately and together are described. When a mixture of the two fractions is heated a product is formed with properties different from those of either fraction heated separately. This product is a complex formed by the two fractions uniting as they undergo denaturation.

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STUDIES ON THE FEEDING METHODS AND PENETRATION RATES OF *MYZUS PERSICAE* SULZ., *MYZUS CIRCUMFLEXUS* BUCKT., AND *MACROSIPHUM GEI* KOCH.

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With Plates 14 and 15

EXPERIMENTS on the transmission of three strains of *Hyoscyamus* virus III, potato virus Y, and cucumber virus I by the aphid species *Myzus persicae* Sulz., *M. circumflexus* Buckt., and *Macrosiphum gei* Koch. (Watson, 1938; Watson & Roberts, 1939) have shown that most infections are obtained after previously fasted aphides have fed for only 2 min. on the source of infection. Infectivity decreases rapidly when the infection feeding time is increased to 15 min. and still further to 3-5 hr.

Though this response to short infection feeding periods is common to the three aphides with the viruses under discussion there are slight variations in the efficiency of the different aphid species with some of the viruses. It was hoped, therefore, that a study of the feeding habits and penetration rates of these aphides on tobacco might throw light on the cause of the variations.

No decrease in the numbers of infections with increased feeding periods of *Myzus persicae* such as are described above were obtained with Sugar Beet Yellow virus. The feeding methods of this aphid on sugar beet, therefore, were also investigated to see whether any explanation could be found for the different relationship between insect and virus observed for Sugar Beet Yellows and viruses of the *Hyoscyamus* III type.

MATERIAL AND METHODS

The aphides used were from cultures of *Myzus persicae*, *M. circumflexus*, and *Macrosiphum gei* kept in stock as partheno-genetic, apterous, viviparous females. They were from the same stock as those used in the insect transmitting experiments (Watson & Roberts, 1939). The plants used were seedlings of White Burley tobacco, and Kleinwanzleben E sugar beet.

Aphides, previously collected and starved were placed in batches of about thirty on young leaves supported in wet sand under a bell jar to ensure humid and undisturbed feeding conditions. After the required feeding time the leaves were plunged into a shallow dish containing boiling Schaudinn's fluid. After a few seconds they were transferred to absolute alcohol where the sections of leaf to which aphides still adhered were cut out in sizes convenient for embedding. These sections were fixed for $\frac{1}{2}$ hr. in Carnoy's fluid, washed in a solution of iodine in absolute alcohol, and again in fresh absolute alcohol, and transferred by gradual stages through cedar-wood oil to paraffin wax with a melting point of 56°.

Although many aphides were lost by this method, especially in the Schaudinn's fluid, no other was found which gave equally rapid and satisfactory fixation for both insects and leaf tissue. Loss of aphides took place chiefly in the short feeding periods; for example, out of 164 *Myzus circumflexus* fed for 5 min. on tobacco leaves, forty were finally embedded in wax, and of these six gave stained and mounted preparations showing the stylets in place in the tissues. In the short feedings of the aphid *M. persicae*, which had not previously been starved, the percentage of success was even less. In one

batch of approximately 370 aphids fixed, fifty-eight were embedded in wax, and only four successful mounted preparations were obtained.

Most of the sections were cut at 20μ , as thinner sectioning rarely gave the complete path taken by the stylets in one or two serial sections. The stylets were often broken and moved in cutting and a more accurate indication of the depth they had penetrated was given by the stylet tracks. Measurements were made only where the stylet track was clearly visible, and were taken from the point of entry of the stylet to the end of the stylet track. Curves were disregarded, so in some cases the figures given in the tables are not the actual length of stylet in the leaf tissue.

Sections were stained in safranin and light green, both in 95 % alcohol, and mounted in canada balsam.

Some of the data included here on the penetration rates of *M. persicae* on tobacco are from preparations made by Mrs W. Cochran (Mitchell, 1937) in which a different fixing technique was used. By this method boiling absolute alcohol was poured over the aphides feeding on leaves in shallow dishes. The leaves with the insects attached were then cut up, further fixed in fresh absolute alcohol, transferred gradually to cedar wood oil, and embedded.

FEEDING HABITS AND PENETRATION RATES OF APHIDES

(1) *Myzus persicae* on tobacco

Aphides were fixed on tobacco at feeding periods of 5, 15, and 60 min. The majority had previously been starved, though in Table 1 data are included on some previously unstarved insects in the 5 min. feeding period. Previous experiments showed (Watson & Roberts, 1939) that for all aphides and viruses infectivity increased considerably with increasing time of preliminary fasting.

Reference has previously been made to the difficulty of fixing aphides in the unstarved group, possibly because the latter take longer to "settle" on the leaves than the fasting aphides. Data on their penetration rates, therefore, are scanty. In Table 1, however, comparison between four unstarved and four starved aphides (*M. persicae*) shows that the average depth of penetration is slightly more for the starved insects. This difference, however (between 45 and 52μ), is not thought to have any bearing upon the difference in infectivity between the starved and unstarved insects. The average depth penetrated by the four starved and four unstarved aphides is 49μ .

The results obtained by Mitchell (1937) for 5 min. feeding periods gave a deeper average penetration of 63μ . For purposes of comparison with the short feeding periods of *M. circumflexus*, therefore, the average depth of penetration from my own preparations only will be taken into consideration in order to ensure uniformity in the method of measurement.

Table 1 shows that none of the fifteen aphides fed for 5 min. had penetrated as far as the phloem though two had reached cells adjoining it. The great majority had penetrated through the epidermis usually only as far as the first or sometimes the second cell of the mesophyll (Pl. 14, figs. 1, 2). Even in the 15 min. feedings only one insect reached the phloem, and one the cells adjoining it. After 1 hr. feeding five out of nine, or 55 %, of the aphides were feeding in the phloem, and two of the remaining four were near it. Thus at the period when the highest number of successful infections are obtained, i.e. after 5 min. on the infected plant, the majority of the aphides have penetrated only just below the epidermis.

59 % of the penetrations by *M. persicae* were intracellular. 28 % were intercellular and of these only one had penetrated through a stoma (Pl. 14, fig. 1).

TABLE 1. *Method of feeding and penetration rates of Myzus persicae on tobacco*

Feeding time	Slide no.	Method of penetration			In phloem	Not in phloem	Depth of penetration (μ)
		Intra-cellular	Inter-cellular	Doubtful			
5 min. (unstarved aphides)	1	+	.	.	.	+	46
	2	.	.	+	.	+	44
	3	+	.	.	.	+	56
	5	.	+	.	.	+	36
	Total	4	2	1	0	4	Mean 46
5 min. (starved aphides)	1	+	.	.	.	+ ^c	90
	2	+	.	.	.	+	38
	3	+	.	.	.	+	54
	4	.	+	.	.	+	24
	Total	4	3	0	0	4	Mean 52
5 min. (starved aphides)	*1	+	.	.	.	+ ^c	100
	*2	.	+	.	.	+	11
	*3	+	.	.	.	+	66
	*4	.	+	.	.	+	44
	*6	+	.	.	.	+	144
	*8	+	.	.	.	+	42
	*9	+	.	.	.	+	36
	Total	7	5	0	0	7	Mean 63
Total for all 5 min. feedings	15	10	4	1	0	15	
%		67	27	7	0	100	
15 min.	*10	+	.	.	.	+	42
	*11	.	+	.	.	+	56
	*12	+	.	.	.	+	6
	*13	+	.	.	.	+	156
	*14	+	.	.	.	+ ^c	96
	*15	.	+	.	.	+	112
	*16	+	.	.	+	.	102
	*17	+	.	.	.	+	32
	Total	8	6	2	0	1	7 Mean 75
%		75	25	0	13	88	
1 hr.	*18	.	+	.	.	+	60
	*19	.	.	+	.	+ ^c	96
	*20	.	.	+	+	.	104
	*21	+	.	.	+	.	206
	*22	.	+	.	+	.	200
	*23	+	.	.	.	+ ^c	90
	*24	.	.	+	+	.	210
	*25	+	.	.	.	+ ^c	142
	*26	.	+	.	+	.	176
	Total	9	3	3	5	4	Mean 143
%		33	33	33	56	44	
Total	32	19	9	4	5	27	
%		59	28	13			

* From Mitchell (1937); ^c near phloem; ^s penetration through stoma. N.B. Measurements in first eight slides made in straight line from point of entry of stylets to end of stylet track.

(2) *Myzus circumflexus* on tobacco

Aphides were fixed feeding on tobacco after the following periods: 5 min. and 1, 1½, and 24 hr.

The average depth of leaf penetrated by this insect (Table 2) after 5 min. feeding was 57 μ compared with 49 μ by *M. persicae* (Pl. 14, figs. 3, 4a and 4b). None of the short period feedings was successful in reaching the phloem. Only one out of fifteen aphides had penetrated as far as the centre of the leaf and this was not in the vicinity of a vascular bundle.

TABLE 2. *Method of feeding and penetration rates of Myzus circumflexus on tobacco*

Feeding time	Slide no.	Method of penetration			In phloem	Not in phloem	Depth of penetration μ
		Intra-cellular	Inter-cellular	Doubt-ful			
5 min.	1	+	.	.	.	+ ^d	110
	2	.	+ ^s	.	.	+	26
	3	.	.	+	.	+	.
	4	.	+	.	.	+	60
	5	.	+	.	.	+	62
	6	.	.	+	.	+	60
	7	.	+	.	.	+	30
	8	.	+	.	.	+	40
	9	.	.	+	.	+	.
	10	.	+	.	.	+	54
	11	.	+ ^s	.	.	+	26
	12	.	+	.	.	+	110
	13	.	+ ^s	.	.	+	54
	14	.	.	+	.	+	.
	15	.	+	.	.	+	50
Total	15	1	10	4	0	15	Mean 57
%		7	67	27	0	100	
1 hr.	1	.	.	+	+	.	110
	2	.	+	.	+	.	112
	3	+ ^s	.	.	+	.	90
1½ hr.	1	.	.	+	+	.	.
	2	.	.	+	+	.	.
	3	.	+	.	+	.	110
Total	6	1	2	3	6	0	Mean 105
%		17	33	50	100	0	
24 hr.	1	.	+	.	+	.	102
	2A	+	.	.	+	.	180
	2B	.	+	.	.	+	146
	3	.	+	.	.	+	190
	4	.	+	.	+	.	170
	5	+	.	.	+ ^b	.	126
	6A	.	+	.	.	+ ^c	138
	6B	.	+	.	.	+	44
	7	.	+	.	+	.	190
	8	.	+	.	+	.	196
Total	10	2	8	0	6	4	Mean 147
%		20	80	0	60	40	
Total	31	4	20	7			
%		13	64	23			

^b In xylem; ^c near phloem; ^d deep enough, but not in vicinity of vascular bundle; ^s penetrated through stoma.

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In the 1-1½ hr. feeding group only six aphides were fixed and all had succeeded in reaching the phloem. In the 24 hr. group 60% were feeding in the phloem; this group includes one aphid which had withdrawn its stylets from the phloem, and was feeding in the xylem (Pl. 14, figs. 5, 6). The remainder had, for the most part, penetrated the leaf sufficiently deeply, but not in the vicinity of a vascular bundle.

The most striking difference between *M. circumflexus* and *M. persicae* lies in the fact that 64% of the penetrations by the former were intercellular (Table 5). 13% were intracellular, and 23% doubtful (Pl. 14, figs. 4, 5). Penetration through the stomata also appears to be more frequent than with *M. persicae* (Table 5).

(3) *Macrosiphum gei* on tobacco

Aphides were fixed at feeding periods of 1 and 24 hr. No stomatal penetrations were observed (Table III).

TABLE 3. *Method of feeding and penetration rates of Macrosiphum gei on tobacco*

Feeding time	Slide no.	Method of penetration			In phloem	Not in phloem	Depth of penetration μ
		Intra-cellular	Inter-cellular	Doubt-ful			
1 hr.	2	+	.	.	+	.	80
	3	+	.	.	+	.	84
	4	+	.	.	+	.	120
	5	+	.	.	+	.	120
	6A	+	.	.	.	+	50
	6B	+	.	.	.	+	56
	7	+	.	.	+	.	66
	8	+	.	.	+	.	130
Total	8	8	0	0	6	2	Mean 88
%		100	0	0	75	25	
24 hr.	1	+	.	.	.	+ ^d	104
	2A	+	.	.	+	.	160
	2B	+	.	.	+	.	160
	2C	+	.	.	+	.	164
	3	+	.	.	+	.	140
	4	+	.	.	+	.	210
Total	6	6	0	0	5	1	Mean 156
%		100	0	0	83	17	
Total	14	14	0	0	11	3	Mean 122
%		100	0	0	79	21	

^d Deep enough, but not in vicinity of vascular bundle.

Compared with *Myzus persicae* and *M. circumflexus*, *Macrosiphum gei* was remarkable for the consistency shown by it in the intracellular method of penetration, and for the high percentage of aphides actually feeding in the phloem tissue (Pl. 14, fig. 7). Its performance in these respects is compared with that of the other aphides in Tables 4 and 5.

According to these observations a very high percentage of *M. gei* feed in the vascular tissue. This differs from the findings of Dykstra & Whitaker (1938) with *Macrosiphum (Illinoia) solanifolii* (probably synonymous with *M. gei*) on potato. These workers suggested that the reason why this aphid was a less efficient vector of leaf roll than *Myzus persicae*,



Fig. 1.



Fig. 2.



Fig. 3.



Fig. 4a.



Fig. 4b.



Fig. 5.

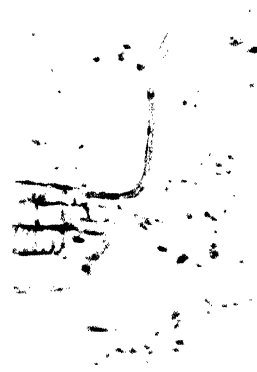


Fig. 6.



Fig. 7.



Fig. 8.

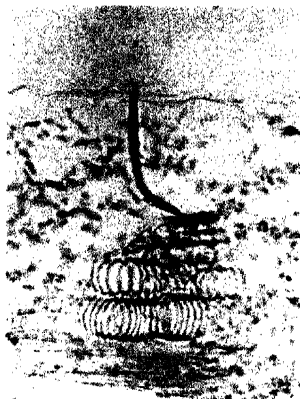


Fig. 9.

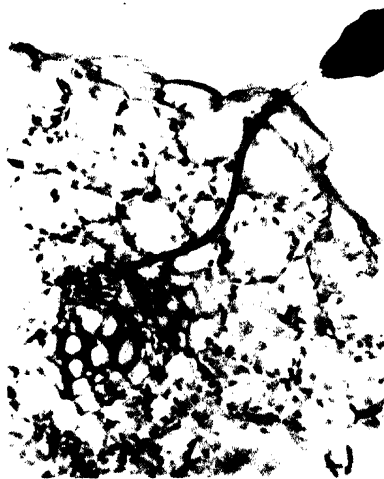


Fig. 10.



Fig. 11.



Fig. 12.

M. circumflexus, and *M. solani* was because it was the only one of the four studied which fed less than 50% of the time in the vascular region.

TABLE 4. *Aphides feeding in the phloem after 1-24 hr. Comparison between Myzus persicae, M. circumflexus, and Macrosiphum gei*

Aphides	Total no. of aphides	Feeding in phloem	%
<i>Myzus persicae</i> on tobacco	9	5	55
<i>M. persicae</i> on sugar beet	21	13	62
<i>M. circumflexus</i> on tobacco	16	12	75
<i>Macrosiphum gei</i> on tobacco	14	11	79

TABLE 5. *Comparison between the penetration methods of Myzus persicae, M. circumflexus, and Macrosiphum gei on tobacco, and Myzus persicae on sugar beet*

Aphides	Total no. of aphides	% doubtful	% intracellular	% intercellular	Penetration through stoma
<i>M. persicae</i> on tobacco	32	13	59	28	1
<i>M. circumflexus</i> on tobacco	31	23	13	64	3
<i>M. gei</i> on tobacco	14	0	100	0	0
<i>M. persicae</i> on sugar beet	39	10	59	31	2

Smith (1926) stated that *Myzus persicae*, *M. circumflexus*, and *Macrosiphum solanifolii* (*M. gei*) on potato "appear to favour an intercellular method of penetration" though *M. solanifolii* "occasionally follows an intracellular course to the phloem". Table V shows that the behaviour of these three insects on tobacco and of *Myzus persicae* on sugar beet is contrary to the results obtained by Smith on potato. All the aphides, especially *Macrosiphum gei*, preferred the intracellular method of penetration except *Myzus circumflexus* which penetrated by this method in only 13% of the preparations studied.

(4) *Myzus persicae* on sugar beet

Aphides were fixed on sugar-beet leaves after feeding times of 15 min., and 2 and 24 hr. Table 6 shows that only two out of eighteen aphides succeeded in penetrating the phloem after 15 min. feeding. Some of the unsuccessful aphides had reached cells near the phloem, had penetrated the xylem, or had penetrated the leaf sufficiently deeply though not near a vascular bundle (Pl. 14, figs. 8, 9). Even after 2 hr. only 50% of the insects were feeding in the phloem, though here a higher percentage of the unsuccessful ones had reached the edge of the vascular bundle (Pl. 14, figs. 10, 11).

Tables 4 and 5 show that the feeding habits of *M. persicae* on sugar beet and on tobacco are very similar and, like *Macrosiphum gei*, well over 50% of the penetrations are intracellular. The rate of penetration of *Myzus persicae* on sugar beet and its bearing on the infectivity of the aphides in the transmission of sugar-beet yellows virus will be discussed in a later section.

TOXIC OR OTHER EFFECTS ON THE HOST PLANT

Smith (1926) stated that in the case of *Myzus persicae* the effect of the salivary secretions on the host (potato) is considerable and greater than that of *M. circumflexus* and *Macrosiphum solanifolii* (Ashmead). Adjacent to the path of the stylet track the cell contents are disorganized, the chloroplasts and nuclei frequently being destroyed. He also observed that

TABLE 6. *Method of feeding and penetration rates of Myzus persicae on sugar beet*

Feeding time	Slide no.	Method of penetration			In phloem	Not in phloem	Depth of penetration μ
		Intra-cellular	Inter-cellular	Doubtful			
15 min.	1	.	+	.	.	+a	42
	2	+	.	.	.	+	160
	3	.	.	+	.	+	?
	4	.	+	.	+	.	110
	5	.	+	.	.	+b	60
	6	.	.	+	+e	.	140
	7a	+	.	.	.	+c	86
	7b	+	.	.	.	+	40
	8	+	.	.	.	+	110
	9	.	+	.	.	+d	90
	10	+	.	.	.	+	100
	11	.	.	+	.	+	?
	12	.	+	.	.	+a	130
	13	+	.	.	.	+	84
	14	.	.	+	.	+	?
	15	+	.	.	.	+	?
	16	+	.	.	.	+	84
	17	+	.	.	.	+d	?
Total	18	9	5	4	2	16	Mean 96
%		50	27	23	11	89	
2 hr.	1	.	+	.	+	.	120
	2a	.	+	.	.	+c	140
	2b	+	.	.	+	.	230
	3	+	.	.	+	.	120
	4	+	.	.	+	.	108
	5	+	.	.	+	.	74
	6	+	.	.	+	.	186
	7	.	+	.	.	+c	280
	8	+	.	.	.	+	62
	9a	+	.	.	.	+c	138
	9b	+	.	.	.	+	250
	10	+	.	.	.	+	76
Total	12	9	3	0	6	6	Mean 149
%		75	25	0	50	50	
24 hr.	1	+	.	.	+	.	80
	2	.	+	.	+c	.	220
	3a	+s	.	.	+	.	100
	3b	+	.	.	+	.	80
	4	.	+	.	.	+c	100
	5a	.	+	.	+	.	170
	5b	.	+	.	+	.	180
	5c	+	.	.	+	.	80
	6	+	.	.	.	+	104
Total	9	5	4	0	7	2	Mean 124
%		55	45	0	77	22	
Total	39	23	12	4	15	24	
%		59	31	10			

a, near xylem; b, in xylem; c, near phloem; d, deep enough, but not in vicinity of vascular bundle; s, penetration through stoma; e, through xylem.

"in stem punctures, as in the leaf, the excess of saliva is so great that the epidermal cells and those underlying are entirely destroyed giving rise to a cup-like depression on the stem surface". Wound reaction in the phloem with abnormal division of the cells could sometimes be seen. Toxic reaction to *Myzus circumflexus* and *Macrosiphum solanifolii* by the cells of the host were similar to *Myzus persicae* though slightly less marked.

In this study of *M. persicae* on tobacco and sugar beet, and *Macrosiphum gei* and *Myzus circumflexus* on tobacco no toxic reaction of the host plant to the insects' saliva was observed. Apart from the safranin staining stylet track there did not appear to be any accumulation of excess saliva in the host plant. The reactions observed above may be due to greater sensitivity of the potato to the saliva of insects, or to the fact that none of the aphides discussed here were fed for more than 24 hr. on the host plant. Brandes (1923), however, who fixed colonies of *Aphis maidis* feeding on leaves clipped from the host plant, found no apparent wound reaction on the part of the plant visible either in the phloem or from the outside.

DISCUSSION

Investigation of the feeding habits and penetration rates of three aphides *Myzus persicae*, *M. circumflexus* and *Macrosiphum gei* concerned in the transmission of the three strains of *Hyoscyamus* virus III, and of cucumber virus I and potato virus Y show that efficiency of the vectors does not depend on localization of these viruses in the vascular tissue of the plant such as Bennett (1934) believes to be the case in curly-top disease of beet. In general, infectivity of the aphides does not seem to be associated with any particular tissue tapped during feeding or with any particular method of penetrating the plant. The one possible exception to this is discussed later.

It has been shown (Watson, 1938; Watson & Roberts, 1939) that "an increase in vector efficiency corresponding with an increase in the times for which the aphides fasted before feeding on the infected plants was shown by all the aphides in their transmission of all the viruses" and that the vector efficiency of all the aphides decreased with increasing feeding time on the infected plants if the aphides had previously been starved.

This decrease in efficiency in the case of *Myzus persicae* with *Hyoscyamus* virus III was as follows: of a total of forty-five plants (five plants per treatment repeated on nine occasions) infection feeding of 2 min. resulted in thirty-four infections; 15 min., eighteen infections; and 4 hr., five infections. Watson (1938) suggested that the most likely explanation for the effect of preliminary fasting and infection feeding times is that some substance is produced by the insect when feeding which inactivates the virus or forms an uninformative compound with it. This seems so far to be the only reasonable explanation for the effect that preliminary fasting and short infection feeding have on the infectivity of the aphides.

If the virus were localized in the superficial tissues of the leaf instead of in the phloem this still could not account for the drop in infectivity between 5 and 15 min. nor could it account for the difference between starved and unstarved aphides. Table 1 shows that in the 5 min. feedings of *M. persicae* on tobacco two out of eleven aphides had almost succeeded in reaching the phloem. The others had reached various points between the vascular bundle and the epidermis. If we suppose that in the short feedings the only insects which were fixed on the leaves were particularly rapid or good feeders, there is still not enough difference between the depth penetrated by these and the 15 min. insects (only one of which penetrated the

phloem) to account for a major difference in infectivity on the grounds that the virus might be more easily available to the aphides in the superficial leaf tissues.

Localization of the virus, or its availability to the insect might, however, account for the difference in behaviour between *M. circumflexus* and *M. persicae* in the transmission of cucumber virus I. *M. circumflexus* shows typical decrease in infectivity between 5 and 15 min. feeding. It also shows a higher degree of relative efficiency with this virus than *M. persicae* whereas with both *Hyoscyamus* virus III and potato virus Y it is a less efficient vector than *M. persicae*. When cucumber virus I is transmitted by *M. persicae* there is no decrease in vector efficiency between 5 and 15 min. and on some occasions maximum efficiency is not reached until 15 min. Watson & Roberts (1939) suggested that at the time of optimum infectivity (less than 5 min. for *M. persicae*) the insect has not reached tissues where the virus is most highly concentrated, and that by the time these tissues are reached the virus is already being inactivated by the secretion produced during feeding. Small differences in penetration rates, or partiality of *M. circumflexus* for the virus-infected tissues were advanced as reasons which might enable this aphid to reach these tissues before potential infectivity had been very greatly reduced.

From data presented in Tables 1 and 2 it seems that *M. circumflexus* penetrates more deeply than *M. persicae* during 5 min. feeding, the difference being between 57μ for *M. circumflexus* and 47μ (starved and unstarved aphides) or 52μ for starved *M. persicae* (Pl. 14, figs. 1-4). Too few insects were fixed for any great reliance to be placed on these latter figures but it is possible also that the intercellular method of penetration preferred by *M. circumflexus* is more efficient than the intracellular method of *M. persicae* in reaching the tissues where cucumber virus I is most concentrated.

The problem of the insect transmission of sugar-beet yellows virus, a member of a group which survives in its vector for comparatively long periods, differs from that of viruses such as *Hyoscyamus* virus III, potato virus Y, and cucumber virus I which survive in their vectors always for a shorter period than that during which they remain active in untreated infective plant sap. The names "persistent" and "non-persistent" viruses have been proposed to describe these two groups (Watson & Roberts, 1939).

Unpublished experiments on the transmission of sugar-beet yellows virus by *M. persicae* have shown that the efficiency of the vectors increases with increasing feeding time on infected plants. Very rarely could infections be obtained after 7 min. feeding, and only when the feeding time on the healthy plants was more prolonged. The shortest time of total feeding in which an infection was obtained was 30 min. After $\frac{1}{2}$ hr. on the infected and 24 hr. on the healthy plants, 16% successful infections were obtained. This percentage increased to 66% for 2 hr. and to 82% for 24 hr. infection feeding. The number of aphides which penetrate to the phloem, also increases with increasing feeding time, i.e. after 15 min. 11% of the insects were feeding in the phloem, after 2 hr. 50%, and after 24 hr. 77% (Table 6). These rates of increase for successful infections and for the number of insects feeding in the phloem with increasing infection feeding times are not strictly comparable, as, on the one hand, five aphides were used per plant to obtain a successful infection, and on the other, it is probable that the only aphides fixed were those that had deeply penetrated the leaf. The transmission experiments with sugar-beet yellows virus also showed that there was an independent increase in infectivity of the vectors with increasing feeding times on the healthy plants. Bennett (1934), working on the transmission of curly-top disease of sugar

beet by *Eutettix tenellus* found that few insects became infective by feeding on the non-vascular tissue.

Although there is no proof that sugar-beet yellows virus must be obtained from and inoculated into the phloem, some of the results suggest it. Even when fed for 24 hr. or longer all the aphides do not cause infections, and in all the preparations fixed a few aphides consistently failed to feed in the phloem even after 24 hr. Insects rarely reach the phloem in 15 min. and few infections are obtained from insects fed for similarly short times. If phloem feeding were essential, these observations would explain non-transmissions of the virus. Factors, however, such as the amount of virus ingested by the aphides, and possibly the time during which potential infectivity is retained, undoubtedly contribute to the results obtained in the transmission experiments, and could explain them independently of the feeding behaviour of the aphides in the plant.

SUMMARY

1. The feeding habits and penetration rates of three aphides *Myzus persicae*, *M. circumflexus*, and *Macrosiphum gei* on tobacco, and of *Myzus persicae* on sugar beet were investigated in relation to their transmission of *Hyoscyamus* virus III, potato virus Y, cucumber virus I, and sugar-beet yellows virus.

2. Neither *M. persicae* or *M. circumflexus* were found to reach the phloem after 5 min. feeding on tobacco. Very few *M. persicae* penetrated the phloem in 15 min. on tobacco or sugar beet. Even after 24 hr. a few aphides do not penetrate the phloem but feed on non-vascular tissue.

3. *Myzus persicae* and *Macrosiphum gei* were found to penetrate by the intracellular method in more than 50% of the slides examined, and *Myzus circumflexus* in less than 50%. *Macrosiphum gei* showed a higher percentage of phloem penetrations in 1-24 hr. than the other aphides.

4. There were no visible toxic effects on the part of the host plants to the insects' saliva with either tobacco or sugar beet.

5. A possible correlation between the behaviour of *Myzus persicae* and *M. circumflexus* with cucumber virus I and localization of this virus in the leaf is discussed.

6. Increased infections with increased feeding times on both infected and healthy plants, in the transmission of sugar-beet yellows virus by its vector *M. persicae* is discussed in relation to phloem feeding.

Thanks are due to Mrs W. G. Cochran (B. I. M. Mitchell, Ph.D.) for permission to use data included in Table 1, and to Mrs M. A. Watson for helpful criticism and advice.

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EXPLANATION OF PLATES 14 AND 15

PLATE 14

- Fig. 1. Unstarved *M. persicae* after 5 min. on tobacco; penetration through a stoma. Stylets only. $\times 420$.
- Fig. 2. Unstarved *M. persicae* after 5 min. on tobacco. Stylets and stylet track. $\times 420$.
- Fig. 3. *M. circumflexus*. Depth penetrated after 5 min. on tobacco. Stylets and stylet track. $\times 420$.
- Fig. 4. Deep 5 min. intercellular penetration by *M. circumflexus*: (a) entry of stylets, (b) end of stylet track immediately below centre of picture entering from the right. $\times 420$.
- Fig. 5. Intercellular penetration by *M. circumflexus* after $1\frac{1}{2}$ hr. Feeding in phloem. $\times 420$.
- Fig. 6. *M. circumflexus* after 24 hr. The stylet track can be seen near the phloem. Stylets curved to xylem. $\times 420$.
- Fig. 7. *Macrosiphum gei* after 1 hr. End of stylet track forked. Feeding in phloem. $\times 420$.

PLATE 15

- Fig. 8. *M. persicae* after 15 min. feeding on sugar beet. Penetration to non-vascular tissue. $\times 420$.
- Fig. 9. *M. persicae* after 15 min. feeding on sugar beet. End of stylets in xylem. $\times 420$.
- Fig. 10. *M. persicae* on sugar beet. Stylets in phloem of vascular bundle after 2 hr. feeding. $\times 420$.
- Fig. 11. *M. persicae* on sugar beet. Stylets in phloem after 2 hr. feeding. $\times 105$.
- Fig. 12. *M. persicae* on sugar beet. Stylets have passed through xylem to reach phloem. After 24 hr. feeding. $\times 105$.

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THE CHOICE OF DRINKING WATER BY THE HONEYBEE

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(Received 15 February 1940)

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INTRODUCTION

DURING the spring and early summer months, bees collect large quantities of water for use in the hive for such purposes as softening down winter stores, etc.

Hertz (1935) showed without doubt, by means of standard training table experiments, that the honeybee can find pure water by "scent" alone, i.e. that they can distinguish the presence or relative proximity of water by means of the relative water-vapour content of the air. Her experiments give only an approximate indication of how far the "scent" can be distinguished, since the distance from the water surface to the bees is only one of the factors responsible for the water-vapour content of the atmosphere at that distance. Although she demonstrated that bees are sensitive to the water-vapour content of the air, it should not, of course, be assumed that water is sensed by the bee as a "smell". We have no evidence that indicates that the receptors involved are the same as those involved in the perception of ethereal oils, or even that the process is chemical rather than physical. It is not to be doubted, however, that it is a true perception by means of sense organs situated in some portion of the body of the bee. The experiments of Hertz were not concerned as are the ones about to be described, with the water *requirement* of bees.

It is well known that honeybees tend to collect water from many undesirable sources, such as rain-water gutters that are choked with decaying organic matter, on the puddles that form on the top of cow dung and sewage effluent, rather than from a source of clean water provided in the apiary for their use. It was, therefore, considered to be of interest and importance to discover what it is that attracts bees to such sources of water. Obviously something more than the sense of appreciation of the presence of water, as described by Hertz, is involved. Many observations

and a few experiments had already been made on this subject, and the results, although somewhat conflicting, tended to show that bees prefer saline water to pure water. For instance, Herrod-Hempsall (1931) and Harrison (1932) both produced evidence to show that bees will readily collect water that is nearly saturated with sodium chloride; on the other hand, Betts (1932), Preuss (1919) and others, came to the conclusion that 1.5 % sodium chloride is the greatest concentration palatable to bees and about 0.1 % is the optimum concentration. Most observers, in fact, tend to explain the choice of drinking water by the honeybee in terms of the salt content of that water. There are, however, at least four major factors that are likely to be involved: sight, the water perception sense described by Hertz (1935), a perception of various olfactory substances contained in the water, and a gustatory sense once the water has been reached. External physical factors, such as temperature and degree of illumination, also undoubtedly play their part but have been neglected for the purpose of the present study, as they did not vary very greatly throughout each experiment.

METHODS

In order to make this study a "training table", similar to that used by von Frisch and Hertz in so many of their experiments on the senses of the honeybee, was set up in a sunny part of the apiary early in the spring of 1939. This table was painted all over with a matt black enamel in order to conserve as much as possible the heat of the sun's rays, and thirty-six shallow glass Petri dishes 4 in. in diameter were arranged on it in the form of a square. Each dish contained a small quantity of washed dry sand. The bees were first trained to come to this table by offering them very dilute sugar syrup on the sand in all the dishes. Once the bees became used to visiting the table these dishes were replaced by others in which the sand was kept moistened with distilled water. At the same time as many as possible of the natural sources of drinking water, such as rain-water gutters, in the immediate neighbourhood of the apiary were treated with strong lysol or carbolic in order to repel bees from them, thus compelling most of the bees to visit the table in order to collect the water necessary for the requirements of their colonies. After a few days, when the bees had become accustomed to collecting their drinking water at this source, the experiments proper were commenced. By this time thousands of bees were visiting the table daily if weather conditions were favourable. Six different substances at a time were tested against one another on this table and six dishes of each substance were arranged in the form of a Latin square, thus rendering statistical analysis of the results a simple matter. It was found that this modification of the technique usually used in this type of experiment greatly expedited matters, since the significance of the results obtained in an experiment could be checked immediately and the necessity or otherwise of further repetition determined. Six of the dishes on the table in each experiment contained distilled water, so that all the results obtained could be expressed relative to the honeybees' partiality for this substance.

If the substances to be tested were solid salts they were dissolved in fresh distilled water, and sufficient (10 c.c.) of the substance to be tested was poured into each dish to moisten the sand thoroughly, without allowing any of the solution to remain standing on the surface. The dishes themselves were replaced with clean ones fairly frequently in order to minimize, as far as possible, the chance of a bee selecting a particular dish because of any odour left on it by bees that had previously visited it. The whole table was turned round through 90° at intervals in order that bees should not become conditioned to the position of a particular dish containing some substance they favoured. There is little doubt that the presence of a large number of bees on a dish, perceived by a flying bee both visually and by an olfactory sense, tends to attract others, and to overcome this to some extent the table was frequently cleared of bees throughout the course of an experiment.

The table with the substances to be tested was put out in place of the table with the dishes of distilled water, and every 10 min. a count was taken of the number of bees drinking at each dish. It was found that a bee spends on the average between 3 and 5 min. collecting a load of water, thus by taking counts at 10 min. intervals the same bee was unlikely to be counted twice when on the same journey. Frequently the counting had to be done photographically owing to the large number of bees present at one time and the number of bees alighting and taking off from the dishes.

At the end of an experiment the experimental dishes were again replaced with others containing distilled water, and care was taken to see that there was always abundant distilled water on the table between experiments, since these extended over a period of some weeks.

EXPERIMENTS, RESULTS AND CONCLUSIONS

Two types of experiments were undertaken. First, experiments to determine if the honeybees were seeking water for the substances it might contain in solution and, if so, what those substances were and what was their optimal concentration. Secondly, to try to determine why the honeybee tends to prefer tainted water to pure water, i.e. whether it requires the contained substances or is merely led thither by an olfactory response to these substances.

The following tables clearly show the response of the honeybee to various organic and inorganic salt solutions and to other more complicated substances. These salts were chosen because it was thought that they were likely to be essential to the development of the honeybee and that the water collected might be the chief source of them. In addition, they had all been advocated by practical beekeepers for addition to apiary drinking fountains. As will be seen these two types of experiments overlap to a considerable extent.

In Table I are shown the results obtained in a typical experiment; in this case to determine the preferences, if any, of the honeybee for $N/10$ sodium chloride, $N/10$ ferric chloride, $N/10$ magnesium sulphate, $N/10$ sodium carbonate and $N/10$ sodium phosphate to distilled water. All the experiments were conducted on these lines.

Table I. *Latin square showing the results of a typical experiment carried out continuously between 11 a.m. and 3 p.m. on 18 April 1939*

(The numbers below the substances indicate the number of visits paid to them throughout this period)

Distilled water 99	NaCl N/10 80	FeCl ₃ N/10 20	MgSO ₄ N/10 3	Na ₂ CO ₃ N/10 0	Na ₂ HPO ₄ N/10 2
Na ₂ HPO ₄ N/10 1	MgSO ₄ N/10 5	Distilled water 107	Na ₂ CO ₃ N/10 7	NaCl N/10 76	FeCl ₃ N/10 25
FeCl ₃ N/10 16	Na ₂ CO ₃ N/10 3	Na ₂ HPO ₄ N/10 0	NaCl N/10 88	MgSO ₄ N/10 0	Distilled water 100
MgSO ₄ N/10 1	Distilled water 86	NaCl N/10 91	Na ₂ HPO ₄ N/10 3	FeCl ₃ N/10 22	Na ₂ CO ₃ N/10 1
Na ₂ CO ₃ N/10 9	Na ₂ HPO ₄ N/10 1	MgSO ₄ N/10 0	FeCl ₃ N/10 11	Distilled water 111	NaCl N/10 77
NaCl N/10 85	FeCl ₃ N/10 27	Na ₂ CO ₃ N/10 4	Distilled water 121	Na ₂ HPO ₄ N/10 0	MgSO ₄ N/10 4

The results of various experiments to determine the preference of the honeybee for thirteen different salt solutions are shown in Table II.

Table II. *Showing the preference of the honeybee for various salt solutions as compared with distilled water*

(The substances used in each experiment are arranged in order of preference, and the number of visits to each given)

Substances arranged in order of decreasing preference -- (The numbers below the substances indicate the number of visits paid to them)						Exp. no.
Rain water from choked gutter 218	Distilled water 128	NH ₄ Cl N/10 51	KI N/10 27	CaCl ₂ N/10 13	Na ₂ CO ₃ N/10 6	I
Distilled water 497	MgCl ₂ N/40 209	NaCl N/5 164	MgCl ₂ N/20 135	MgCl ₂ N/10 54	MgCl ₂ N/5 28	II
Distilled water 624	NaCl N/10 497	FeCl ₃ N/10 121	Na ₂ CO ₃ N/10 24	MgSO ₄ N/10 13	Na ₂ HPO ₄ N/10 7	III

From the results shown in Table II it appears that the honeybee prefers distilled water to any of the salt solutions offered. It was considered, however, that it was improbable that the optimal concentrations of these salts, from the bee's point of view, had been used, therefore further experiments were carried out in which the three salts, sodium chloride, ammonium chloride and magnesium chloride, which at deci-normal concentrations the bees had seemed to prefer to others, were each offered at five different concentrations. Similar experiments were also undertaken with potassium iodide, since several beekeepers have claimed that the bees both like and obtain benefit from this substance when it is added to their drinking water. The results of these experiments are shown in Table III.

Table III. *Showing the preference of the honeybee for certain concentrations of sodium chloride, magnesium chloride, ammonium chloride and potassium iodide*

Substances in order of decreasing preference (The numbers below the substances indicate the number of visits paid to them)						Exp. no.
NaCl <i>N</i> /40 966	NaCl <i>N</i> /80 926	NaCl <i>N</i> /20 759	Distilled water 579	NaCl <i>N</i> /160 567	NaCl <i>N</i> /10 387	I
Distilled water 502	MgCl ₂ <i>N</i> /160 496	MgCl ₂ <i>N</i> /80 234	MgCl ₂ <i>N</i> /40 189	MgCl ₂ <i>N</i> /20 111	MgCl ₂ <i>N</i> /10 57	II
NH ₄ Cl <i>N</i> /40 318	NH ₄ Cl <i>N</i> /80 290	Distilled water 263	NH ₄ Cl <i>N</i> /160 249	NH ₄ Cl <i>N</i> /20 210	NH ₄ Cl <i>N</i> /10 106	III
Distilled water 130	KI <i>N</i> /80 48	KI <i>N</i> /160 46	KI <i>N</i> /40 28	KI <i>N</i> /20 21	KI <i>N</i> /10 13	IV

As will be seen from the results given in Table III the honeybee definitely appears to prefer *N*/40, *N*/80 and *N*/20 sodium chloride, in the order stated, to distilled water, and is probably unable to appreciate the difference between *N*/160 sodium chloride and distilled water, but definitely prefers distilled water to *N*/10 sodium chloride. Similarly, the honeybee prefers *N*/40 ammonium chloride and probably *N*/80 ammonium chloride to distilled water, and finds it more difficult to distinguish between distilled water and *N*/160 ammonium chloride, or else does not actively dislike the latter solution.

The honeybee does not appear to distinguish clearly between *N*/160 magnesium chloride and distilled water, but prefers the latter to higher concentrations of this salt. In the case of potassium iodide the honeybee appears quite definitely to dislike all the concentrations of this substance that were offered to it.

The above results appear to indicate that the honeybee in a state of nature does not seek water for the contained salts, since none of the solutions tested with the exception of dilute sodium and ammonium chloride solutions had any particular attraction.

Table IV. *Showing the preference of the honeybee for rain water, cow-dung water and urine and some of their constituents over distilled water*

Substances in order of decreasing preference → (The numbers below the substances indicate the number of visits paid to them)						Exp. no.
Rain-water distillate 678	Rain-water residual salt 343	Distilled water 318	Rain-water distillate + charcoal 316	NaCl N/10 223	NaCl N/5 95	I
Cow-dung water distillate 396	Cow-dung water 371	Cow-dung water distillate + charcoal 243	Distilled water 198	NaCl N/10 132	Cow-dung water residual salts 31	II
Urine distillate 737	Distilled water 408	NaCl N/10 282	NH ₄ Cl N/10 166	Urine 93	Urine residual salts 21	III

In the next series of experiments, the results of which are shown in Table IV above, direct attempts were made to discover what causes the honeybee to tend to prefer rain water from a leaf-choked gutter, water that has collected on top of a cow dung and urine to a source of clean water provided in the apiary. That this preference does exist has been reported by many observers. It has also been noted many times in the apiary in which this work was carried out. An instance of bees much preferring rain water from a gutter choked with decaying organic matter to pure water is shown experimentally in Table II, Exp. 1. For the purpose of these experiments large samples of the three substances mentioned above were collected. A sample of each was immediately evaporated to dryness over a very gently heated sand-bath, the distillate being condensed and collected. The minimum of heat was used, since it was regarded as most important to try to prevent the decomposition by heat of any of the contained substances. The residual salts thus produced were carefully redissolved in a bulk of distilled water equal in volume to the amount of the substance (500 c.c.) originally taken. All solutions were carefully filtered at atmospheric or slightly reduced pressure. These two solutions, the residual salt solution and the distillate, produced from these three substances were then offered to the bees together with distilled water. In every case the bees showed a marked preference for the distillate over distilled water. Only in the case of the rain water, where the salt concentration was presumably very low, was the residual salt solution preferred to distilled water.

In the experiments with the urine and the cow-dung water, the original substances were also offered alongside their derivatives. The bees showed a marked preference for the cow-dung water over the distilled water, but, in the case of the urine the bees flew over it in a large group but very few of them collected it. This is in complete accordance with observations made in the field. Although bees have been observed to be strongly attracted by urine they will seldom collect it, unless it is considerably diluted. A few experiments were commenced using different

dilutions of urine, but the relatively short period of the season during which large numbers of bees collect water prevented their completion. So far as they went, however, they definitely tended to show experimentally that bees will collect urine that has been considerably diluted in preference to distilled water. The same thing seems to be true of cow-dung water, although the experiment given in Table IV does not show it. Field observations, however, showed that although bees are often strongly attracted to small puddles of water that collect on the top of cow dung they would only collect it from the older pats, by which time it had, presumably, become more dilute. The cow-dung water used in these experiments was collected from old pats that had been exposed to the weather for about 10 days or more.

An interesting feature of these experiments was the result obtained when samples of rain-water distillate and also cow-dung water distillate were allowed to stand with animal charcoal for 36 hr. at room temperature. When the resulting supernatant fluid was offered to the bees it appeared in the case of the rain water that the bees were no longer able to distinguish between it and distilled water, and in the case of cow-dung water distillate the preference was very markedly reduced. It would therefore appear that some olfactory substance or substances contained in the distillates produced from rain water, cow-dung water and urine, is the factor responsible for attracting the bees to these substances in preference to distilled water. Further, in the case of the rain-water sample and to a large extent the cow-dung water sample, these volatile substances can be absorbed on animal charcoal, in which case the resulting supernatant fluid is found to have lost its strong attraction for the honeybee. The conclusion can therefore be drawn that the honeybee is, in nature, attracted to these sources of water supply by the combined influence of the water perception sense described by Hertz (1935) together with a true olfactory sense. Sight may in nature also play a part in water discrimination, though the possible operation of this sense was, so far as possible, eliminated in the experiments described above. Once the honeybee has found a source of drinking water that it prefers to others in the immediate vicinity of its colony, by means of these senses, it is probably kept there by a gustatory sense, since it has been shown that the honeybee prefers sundry dilute salt solutions to pure water. It is almost certain that in this way a complicated conditioned reflex is set up, an expression of the so-called "memory" of the honeybee, which causes it to visit one particular source of water many times in preference to all others. That this conditioned reflex can persist over a considerable period of time is clearly demonstrated by the fact that honeybees will in the spring visit the site of a drinking fountain, that they used in the previous autumn, even though the fountain had been removed.

Table V, shown above, gives a final summary of the order of preferences of the honeybee for various solutions. If an arbitrary factor 10 is used to express the degree of appreciation of distilled water shown by the honeybee, the degree of preference shown for all other solutions used in these experiments can also be expressed by means of a factor. This factor has been calculated by taking the mean number of visits paid in all experiments to each substance and dividing this by the mean

Table V. *Showing the order of preference of various drinking waters. The various drinking waters have also been given a factor which shows the degree of preference compared with distilled water which has been given the arbitrary factor 10*

Substance	Factor
Rain-water distillate	21
Cow-dung water distillate	20
Cow-dung water	19
Urine distillate	18
Rain water from gutter	17
NaCl N/40	
NaCl N/80	16
NaCl N/20	13
NH ₄ Cl N/40	12
Cow-dung water distillate + charcoal	
Rain-water residual salts	11
NH ₄ Cl N/80	
Rain-water distillate + charcoal	10
MgCl ₂ N/160	
NaCl N/160	
Distilled water	
NH ₄ Cl N/160	9
NH ₄ Cl N/20	8
NaCl N/10	7
MgCl ₂ N/80	5
NH ₄ Cl N/10	4
MgCl ₂ N/40	
KI N/80	
KI N/160	
NaCl N/5	3
NH ₄ Cl N/5	2
MgCl ₂ N/20	
FeCl ₂ N/10	
Urine	
Cow-dung residual salts	
KI N/40	
KI N/20	
KI N/10	1
MgCl ₂ N/10	
Urine residual salts	
CaCl ₂ N/10	0
Na ₂ CO ₃ N/10	
MgCl ₂ N/5	
MgSO ₄ N/10	
Na ₂ HPO ₄ N/10	

number of visits paid in the same experiments to distilled water—the figure for sodium chloride N/40 calculated in this way comes to approximately 1·7. Both sides of the equation were then multiplied by 10 which gives distilled water a factor of 10 in each case, and in the above example gives sodium chloride N/40 a factor of 17. In this way, for example, the preference shown by the honeybee for N/40 sodium chloride solution to distilled water can be expressed reasonably accurately by the following ratio:

$$\frac{\text{N/40 NaCl}}{\text{Distilled water}} : \frac{17}{10}$$

Several of the experiments have been statistically analysed and differences between substances found to be very significant. Though the remaining experiments have not been fully analysed, inspection of the figures shows most of the differences to be very clear cut, and there is little doubt of their significance.

SUMMARY

A brief review of the literature on this subject is given. By means of "training table" experiments in which use was made of the Latin square system in order to allow of rapid statistical analysis of the results obtained, it was shown that:

(1) The honeybee prefers dilute sodium chloride and ammonium chloride solutions to distilled water.

(2) It does not prefer concentrations of these salts higher than $N/20$ solutions and solutions of various other salts to distilled water.

(3) The honeybee appears unable to distinguish between $N/160$ sodium chloride or $N/160$ ammonium chloride and distilled water.

(4) The honeybee is probably largely attracted to such sources of drinking water as rain water from gutters choked with decaying organic matter, sewage effluent, etc.: by a water perception sense coupled with an olfactory appreciation of various volatile substances contained in these sources of water.

(5) The volatile substances present in the distillates from the various naturally occurring solutions examined could be absorbed on to animal charcoal to a large extent, in which case the resulting supernatant fluid was found to have lost its great attraction for the honeybee and was no longer clearly distinguished from distilled water.

(6) The salts contained in these sources of drinking water do not appear to play an important part in attracting the bee thither.

(7) It was found possible to express the preference shown by the honeybee for various solutions by means of numerical factors based on distilled water having an arbitrary factor of 10. Forty different solutions have been arranged in order of preference by this means.

I am greatly indebted to the Statistical Department, Rothamsted Experimental Station, for assistance in analysing the results obtained in these experiments. I should also like to take this opportunity of thanking my predecessor, Mr D. M. T. Morland, who suggested that I should undertake an investigation of this problem, and my colleagues in the Rothamsted Entomological and Bee Research Laboratories.

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A STUDY OF THE FREQUENCY WITH WHICH HONEYBEES VISIT RED CLOVER (*TRIFOLIUM PRATENSE*), TOGETHER WITH AN EXAMINATION OF THE ENVIRONMENTAL CONDITIONS

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(With 4 Text-figures)

THE honeybee plays an important role in the pollination of fruit, vegetable and other crops. This was demonstrated during the years immediately following 1918 when fruit crops in particular were very poor, after the epidemic of acarine and other bee diseases had reduced the population of honeybees in the country by at least 90%. There is little doubt that the honeybee is of much greater value to the nation as a pollinator than as a honey producer, and it is considered by many authorities to be the most valuable of the insect pollinators. An estimation of its value as compared with other insects as a pollinator of various crops is important, so that steps may be taken to increase the population in those localities where there is a deficiency or where the presence of bees is most desirable.

The present paper is concerned with the disputed question of the value of the honeybee as a pollinator of red clover grown for seed. Much work has already been done, notably in America and Holland, in determining the relative value of various insects as pollinators of red clover, a good example of a self-sterile leguminous plant. Little work has, however, been carried out in the British Isles, and it is desirable that observations made in other countries should be repeated and extended in Britain since climatic factors may greatly influence the pollination of plants both by affecting the plants themselves and also the insects responsible for their pollination. The present work was carried out during 1939 and 1940 in two large fields of mixed varieties of red clover growing near Harpenden on a heavy clay soil, the nearest hives being about 400 yd. away.

THE AVAILABILITY OF RED CLOVER POLLEN TO THE HONEYBEE

Parker (1925) showed that the pollen of various plants becomes available to visiting insects at different, but usually definite, times of the day, e.g. the pollen of alsike clover (*Trifolium hybridum*) is available to the honeybee at Ames, Iowa, from 8 a.m. to 6 p.m., whereas from white sweet clover (*Melilotus alba*) pollen is available only during the middle of the day and the early afternoon. Information is needed as to the times of the day at which the pollen of red clover (*Trifolium pratense*) is available to insects in this country, since the honeybee only works under certain environmental conditions, and these conditions and the availability of pollen must coincide to some extent if it is to play any part in the pollination of this plant.

To determine at what time of day the pollen of this plant becomes available for pollination, use was made of the fact that individuals of many species of bumble-bee can be found working red clover throughout the hours of daylight. On a number of days, bumble-bees

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were captured in the clover field, their pollen loads, if present, removed and the insect immediately released and kept under close observation for as long as possible. In this way it was possible to determine whether or not the particular insect was collecting pollen, by seeing whether pollen was loaded into the pollen baskets while the insect was flying from flower to flower and whether the pollen load increased in size. Frequently the bumble-bee

TABLE 1. *The times of day during which pollen was collected from red clover (Trifolium pratense) by bumble-bees at Harpenden, Herts, on 16 June 1940*

No. of bumble-bee	Time under observation (G.M.T.)	Whether collecting pollen or not as determined by	
		Behaviour	Increase in size of load
1	7.15 a.m. - 7.27 a.m.	-	-
2	7.30 - 7.39	-	-
3	7.45 - 8.2	-	-
4	8.15 - 8.22	+	-
5	8.26 - 8.44	+	+
6	9.4 - 9.21	+	+
7	9.29 - 9.35	+	+
8	9.42 - 9.53	+	+
9	10.5 - 10.15	+	+
10	10.48 - 11.17	+	+
11	11.20 - 11.26	+	+
12	11.47 - 12.3 p.m.	+	+
13	12.20 p.m. - 12.33	+	+
14	12.37 - 12.44	+	+
15	12.46 - 12.59	+	+
16	1.5 - 1.8	+	+
17	1.16 - 1.31	+	+
18	2.10 - 2.14	+	+
19	2.17 - 2.39	+	+
20	3.23 - 3.42	+	+
21	4.5 - 4.16	+	+
22	5.0 - 5.18	+	+
23	5.25 - 5.46	+	+
24	5.51 - 6.15	+	+
25	6.24 - 6.37	+	+
26	6.42 - 7.0	+	+
27	7.3 - 7.21	+	+
28	7.26 - 7.44	+	+
29	7.47 - 8.1	+	+
30	8.9 - 8.25	+	+
31	8.37 - 8.42	+	+
32	8.50 - 9.10	+	+
33	9.23 - 9.38	+	+
34	9.47 - 9.52	+	-
35	10.0 - 10.7	-	-
36	10.15 - 10.34	-	-
37	10.42 - 10.46	-	-

on being released after removal of its pollen load flew away and could not be kept under observation. Table 1 shows the results of a typical day's observation. Red clover pollen was available for collection from about 9 a.m. to 8.30 p.m. (G.M.T.) on 16 June 1940. It is probable that pollen was only available in the right condition for transference to the bee's body during this period, otherwise pollen would automatically have been collected at other times by the nectar-seeking bumble-bees.

Other observations were made by noting throughout the day the number of bumble-bees out of ten observed on each occasion in the clover field, carrying out the movements indicative of the transference of pollen to the pollen baskets. Two series of observations were

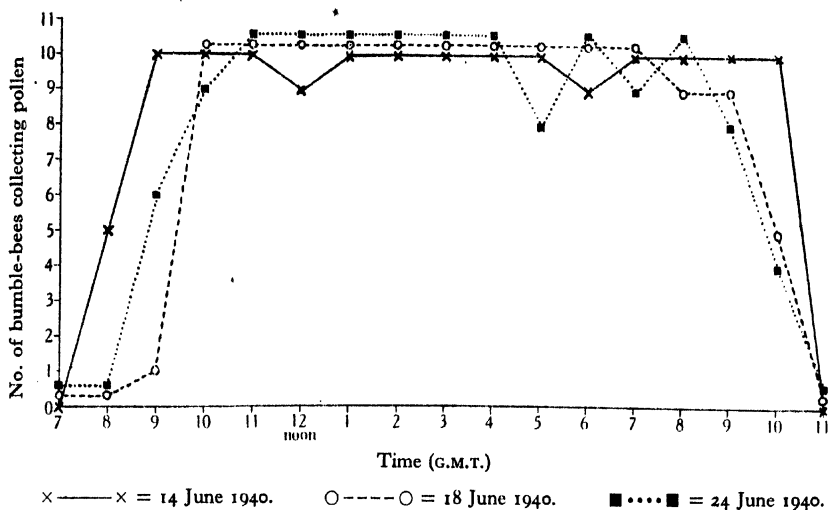


Fig. 1. No. of bumble-bees, in a group of 10 observed on each occasion, collecting red clover pollen on 3 days in June 1940

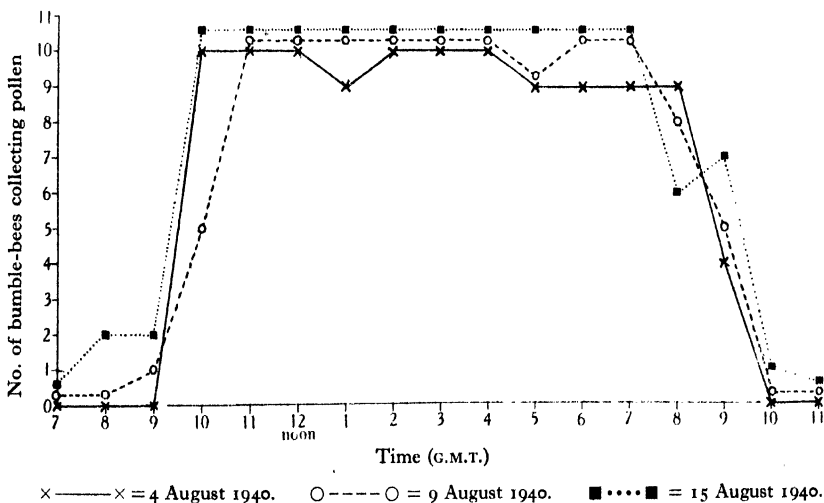


Fig. 2. No. of bumble-bees, in a group of 10 observed on each occasion, collecting red clover pollen on 3 days in August 1940

made, (1) during the first crop of flowers in June, and (2) in August when the second crop of flowers were in bloom, the first having been cut and harvested. The results, expressed in Figs. 1 and 2, are in close agreement with those of Table 1.

THE COLLECTION OF RED CLOVER POLLEN BY THE HONEYBEE

Parker (1926) concluded that, with clovers, except red clover, the pollen is simply collected as a supplementary load while the bee is gathering nectar, and that no special effort in the collection of the pollen is made except to pack it into the pollen baskets and carry it to the hive. With *Trifolium pratense*, however, the bees merely treated this clover as a source of pollen. This does not accord with the work of Dunham (1939), Armstrong & Jamieson (1940) or many other observers (e.g. Valle, 1935; Stapel & Erikson, 1936), which showed that the honeybee does on occasion visit red clover flowers for nectar as well as for pollen. That the honeybee visits even first crop red clover for nectar appears to be supported by the following observations.

The honeybee when going deliberately to collect pollen from a plant carries honey from the hive to moisten the pollen in order to facilitate packing in the pollen baskets; and, when gathering pollen from a plant which has both nectar and pollen available, it invariably uses nectar from the plant itself with which to moisten the pollen. A simple test was applied to honeybees and bumble-bees working red clover at various times to determine whether the contents of the honey-stomach were honey or nectar, i.e. whether the collection of pollen was incidental to the collection of nectar or deliberate. The bee was killed, the contents of the honey stomach absorbed on to filter paper, and the outline of the liquid on the filter paper pencilled in while the drop was still moist. After drying at room temperature for 12 hr., honey leaves a translucent spot, while the nectar spots vary in degree of translucency from almost none to about half that of honey, depending upon the density of the nectar. By comparison with test spots it was relatively easy to decide whether a particular sample was nectar or honey. Of the 484 honeybees examined 277 were carrying nectar, 185 were carrying honey and 22 cases were doubtful, 19 of the latter not containing sufficient fluid for a proper test to be made. Similarly, of 204 bumble-bees examined, all except three which had almost empty honey stomachs, were carrying nectar. It may therefore be concluded that in the Harpenden district during 1940 the honeybee worked red clover for nectar (60%), pollen being collected only incidentally in these cases, and also deliberately for pollen (40%).

 SIZE OF LOAD OF RED CLOVER POLLEN CARRIED BY THE HONEYBEE
 AND NUMBER OF FLOWERS VISITED

In order to obtain a fairly accurate estimate of the number of pollen grains collected by the honeybee on one foraging trip, bees carrying red clover pollen were collected as they entered the hive. The pollen loads were carefully removed from the hind legs of each bee and placed in glycerine. The grains were counted in suspension in glycerine in a Thoma cell after having been prepared by Dunham's method (1939*b*) except that the pollen grains were stained with fuchsin instead of brilliant green. In each instance a check was made to ensure that the grains were those of red clover. During these experiments the bees were chiefly working red clover and white Dutch clover, the pollen grains of which are easily distinguishable when stained in fuchsin and mounted in glycerine gelatine. The results of these counts are given in Table 2, which shows that the honeybees carried on the average approximately 284,000 red clover pollen grains per load, whereas Dunham (1939*b*) found the average figure was about 347,500 grains. Dunham also gave figures for the number of

pollen grains contained in unvisited flowers and flowers pollinated by the honeybee. These averaged respectively 1153 and 148, clearly showing the thoroughness of the honeybee in pollen collecting.

TABLE 2. *Number of pollen grains of red clover carried by honeybee*

No. of bee	Total no. of red clover pollen grains in pollen baskets	No. of bee	Total no. of red clover pollen grains in pollen baskets
1	224,000	16	304,000
2	630,000	17	198,000
3	281,000	18	282,000
4	127,000	19	291,000
5	312,000	20	147,000
6	280,000	21	132,000
7	149,000	22	513,000
8	184,000	23	264,000
9	300,000	24	500,000
10	471,000	25	138,000
11	218,000	26	286,000
12	292,000	27	292,000
13	283,000	28	283,000
14	287,000	29	317,000
15	310,000	30	211,000

The minimum pollinating activity of the honeybee in collecting an average load of red clover pollen can be estimated by dividing the average number of pollen grains per bee by the number of pollen grains which the bees removed from the pollinated flower, i.e. approximately 1000 grains. Making use of Dunham's figures for the number of grains in pollinated and unpollinated flowers one obtains the figure of 284 for the number of flowers visited by each bee in collecting a load of pollen, whereas Dunham obtained a figure of 348. Data obtained by Dunham (1939*b*) indicated that approximately 12.5 red clover flowers were visited per minute. This is in close agreement with the results obtained from about fifty observations made at Rothamsted where on the average fourteen flowers were visited per minute. By dividing the total number of flowers visited in collecting a pollen load by the rate of pollinating activity, a figure of 20.3 is obtained, which represents the number of minutes required to collect an average load of pollen. This is close to the figure of 27.8 min. obtained by Dunham. To check this, experiments were made with marked bees. A bee alighting on the hive entrance with a load of red clover pollen was marked with a quick-drying cellulose enamel and immediately liberated. When the bee left the hive again the time was noted and also the time of return if the bee was carrying, as was usually the case, another load of red clover pollen. It was only possible to make twenty-three observations of this kind, and the average length of time taken by the bee from leaving to returning was approximately 24.2 min. Making allowance for the distance to and from the clover field (approximately 400 yd.) and for the fact that the bee might be fatigued from its previous journeys, this accords well with the figure of approximately 20 min. estimated from the previous observations.

CONSTANCY OF THE HONEYBEE WHEN WORKING RED CLOVER

Numerous observations have been recorded which show the remarkable constancy with which honeybees visit flowers of a single species on any one trip. In order to make certain that this constancy is exhibited by the honeybee when working red clover, bees working

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this plant were captured immediately on their return to the hive and their pollen loads removed and subjected to pollen analysis. Individual smears made of these pollen loads were each washed three times with absolute alcohol to remove substances of a fatty nature adhering to their surfaces, stained for 5 min. at room temperature in acid fuchsin in absolute alcohol, rewashed in absolute alcohol and mounted in glycerine-gelatine. Prepared in this way most of the pollen grains assume the fully expanded state with their walls clearly demarcated by the fuchsin, and there is little danger of confusing them with pollen grains from another species. The pollen loads of 100 bees were examined in early August in this way, and, only in seven were *any* grains other than those of red clover found. In these instances only a small number of foreign grains were present except in the case of one bee which had collected almost equal amounts of knapweed (*Centaurea nigra*) pollen and red clover pollen, the former having been collected first and a layer of red clover pollen added on the top. It would therefore appear that the honeybee when working red clover for pollen was usually very constant to this species on any one trip.

TABLE 3. *The number of bees in a group collected on the alighting board of the hive carrying full loads of red clover pollen and white clover pollen on 25 June 1940*

	a.m.					p.m.						
Hour during which bee carrying pollen returned to hive	7-8	8-9	9-10	10-11	11-12	12-1	1-2	2-3	3-4	4-5	5-6	6-7
Total no. of bees whose pollen loads were examined	9	23	24	54	73	62	81	65	72	72	58	46
No. of bees found to be carrying white clover pollen	2	7	8	14	28	38	57	48	23	25	18	11
No. of bees found to be carrying red clover pollen	0	3	5	22	21	10	6	9	30	33	21	8

CONDITIONS UNDER WHICH THE HONEYBEE WORKS RED CLOVER

As stated (p. 128) the honeybee visits red clover in search of either nectar in which case pollen is usually only collected incidentally, or in deliberate search of pollen. Honeybees searching for pollen only were found during 1939 and 1940, to visit red clover at all times of the day when the pollen of this plant was available to them, i.e. between approximately 9 a.m. and 8.30 p.m. G.M.T. Two peak periods of collection were usually noticeable, one about 10.30 a.m. and a higher peak about 3 p.m. According to Parker (1926) most of the leguminous plants, such as clovers, usually reach their peak of pollen dehiscence in the early afternoon, and that of white Dutch clover (*Trifolium repens*) appears to come somewhat earlier than that of red clover; thus competition between these species is reduced. In the early morning bees are frequently found working red clover for pollen; later in the day practically all the pollen gatherers entering the hive were found to be bringing in white clover pollen; and still later in the afternoon red clover pollen once more became the more abundant of the two (see Table 3).

The factors determining the visits of honeybees to red clover inflorescences in search of nectar and incidentally pollen, appear to be somewhat complicated. Scullen showed (Root, 1939) that the sugars in the nectar of a plant must reach a concentration of at least 17 %

before honeybees collect it. Further, honeybees will desert one species of plant in order to work another as the abundance and concentration of their respective nectars varies. Generally speaking, provided the nectars of several species of plants abundant in a given district are all of approximately the same concentration, the greatest number of honeybees will be found working that species in which the nectar is most abundant and most easily obtainable. Almost throughout the summer of 1940 with its long dry periods the concentrations of the nectars of both red and white clover were practically identical, often as high as 60 %, i.e. nearly saturated. Even so, it was frequently observed that numerous honeybees were working the white clover and few the red clover in search of nectar, despite the fact that the red clover was well within the flight range of the bees in the apiaries in which these experiments were conducted, and was probably more abundant than the white clover.¹ It has often been suggested that the corolla tube of first crop red clover is too long for the honeybee to be able to reach the nectar. Dunham (1939*a*), however, pointed out that the distance which a honeybee is able to reach for nectar in the corolla tube may be computed as approximately 7.9 mm., this figure being made up as follows: 6.3 mm. is the average length of tongue of the honeybee, 0.5 mm. the additional distance which a bee of the indicated tongue length can reach, and 1.1 mm. the average depth in the opening of the corolla tube into which a honeybee can thrust the anterior part of the head. The mean length of 500 corolla tubes collected at random in June 1940 was approximately 9.6 mm. as against a figure of 9.5 mm. for the experimental plants used by Dunham (1939*a*).

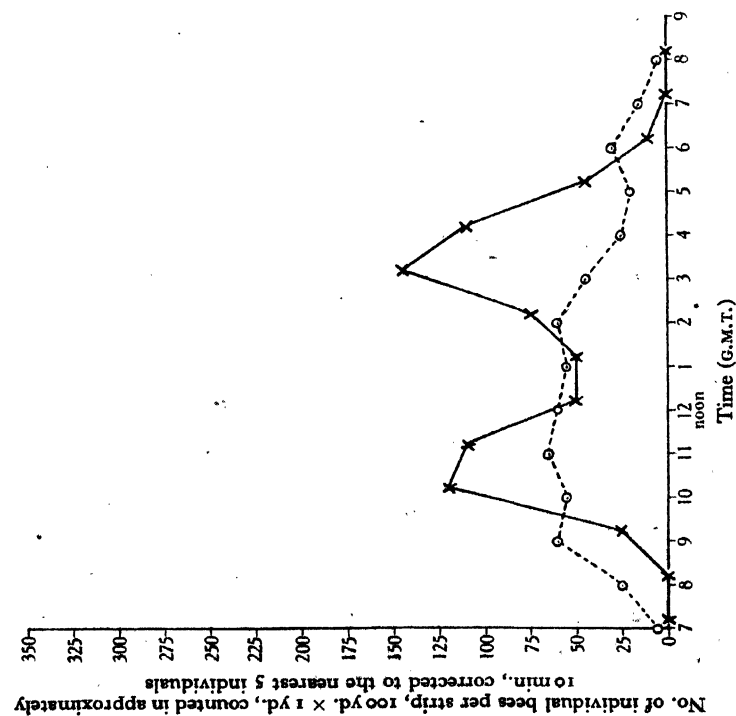
The honeybee can reach the nectar if it rises to 1.7 mm. or more. During the dry summer of 1940 this appeared to happen about once in every 4-5 days usually after a night of heavy dew when the honeybees were found working this plant for nectar. A heavy dew leads to the absorption of water by the nectar and consequently to a rise in height in the corolla tube, which may be sufficient to bring it within reach of the honeybee. A number (127) of measurements were made of the height to which the nectar in the corolla tubes rose, and it was found to vary between approximately 0 and 3.4 mm.

A moderately dry summer with dew at night is more likely to produce conditions under which the honeybee can work red clover for nectar, whereas a wet summer probably causes the plants to grow longer corolla tubes and the nectar to become more dilute.

RELATIVE ABUNDANCE OF HONEYBEES AND BUMBLE-BEES ON RED CLOVER

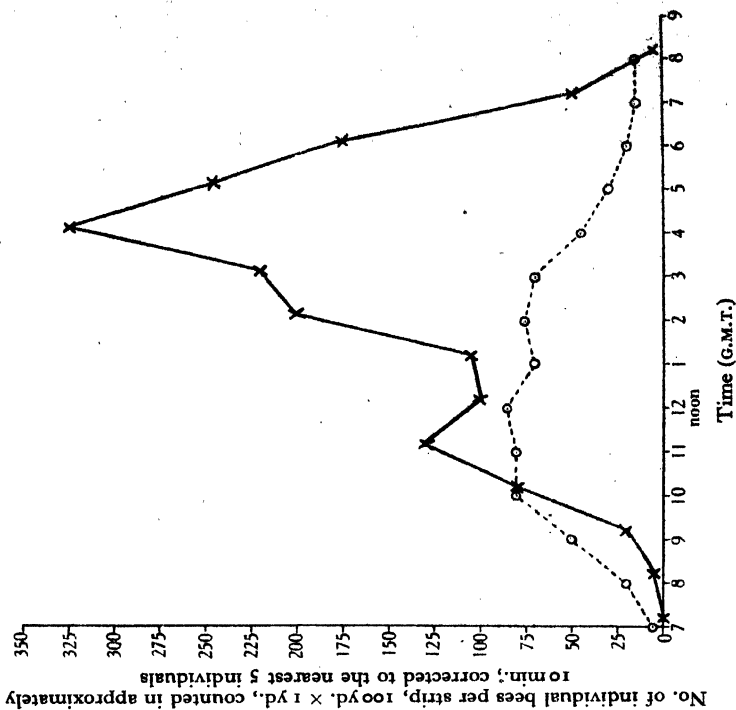
Counts were made at frequent intervals of the number of honeybees and bumble-bees working red clover on a strip 100 × 1 yd. along one side of the field. About 10 min. were taken in walking along the strip making the count. The results obtained during three days in June 1940 when little or no nectar was present in the flowers, and during three days in June when abundant nectar of a high concentration was present are shown respectively in Figs. 3 and 4. When nectar was absent the curve of honeybee activity shows the typical two peaks of pollen collecting activity, but when nectar was present the whole of the days' activity was much greater and the second of the days' peaks is considerably higher.

¹ Analyses of the total sugars present in the nectar samples were made by means of a modification of the Tillmans-Phillipi method (Pirie, 1936) using a Lovibond Tintometer. The amount of nectar required for each test was about 0.5 ml. and was collected by means of a fine capillary pipette thrust into a number of individual flowers. Ten samples were examined at each time and the mean of the results taken.



x ——— x = Honeybees. o ——— o = Bumblebees.

Fig. 3. Mean no. of honeybees and bumblebees counted on a strip of red clover field, 100 yd. \times 1 yd., in approximately 10 min. on 3 days when little or no nectar was present in the flowers in June 1940.



x ——— x = Honeybees. o ——— o = Bumblebees.

Fig. 4. Mean no. of honeybees and bumblebees counted on a strip of red clover field, 100 yd. \times 1 yd., in approximately 10 min. on 3 days when the nectar concentration was very high and the nectar over 2.5 mm. high in the flower tubes in June 1940.

Dunham (1939*a*) in Ohio found that about thirty species of insects are concerned with red clover pollination; that in each year the honeybee was a major factor; and that, with the exception of the bumble-bee, the amount of pollination activity accomplished by other insects was of little value. Although no accurate counts were made general observations strongly suggest that the same is true in this country. Dunham (1939*a*) also measured the relative rate of pollinating activity of six species of bumble-bees and honeybees, and found that with bumble-bees it varied from 16 to 37 flowers per min. and with honeybees from 10.6 to 12.5 flowers per min. Having information on the relative abundance of insects in a given area for each hour of the day for a three-year period, and also their rates of 'pollinating activity', he found it possible to calculate in quantitative terms the relative percentage of pollination performed by honeybees and bumble-bees respectively. Over this three-year period, honeybees could be credited with having performed 82% of the cross pollination and bumble-bees with approximately 15%. The remaining 3% was carried out by other hymenopterous, lepidopterous and dipterous pollinating insects. No comparable data collected in this country are available for making such a calculation; nevertheless since such large numbers of honeybees were found to be present in the clover field at certain times, it would appear likely that the honeybees were responsible for an important part of the pollination of the seed crop.

SUMMARY

Red clover pollen is available from about 9 a.m. to 8.30 p.m. (G.M.T.) in this country.

In the Harpenden district during 1939 and 1940 the honeybee worked first and second crop red clover for both nectar and pollen. Of 484 honeybees working red clover whose 'loads' were examined in detail, 60% were nectar gatherers and 40% pollen gatherers.

The honeybee carries on the average approximately 284,000 red clover pollen grains per load and visits a minimum of 284 flowers in collecting this load. The average time that marked honeybees took when collecting red clover pollen was 24.2 min., which is in good agreement with the calculated figure.

Bees working red clover were remarkably constant to this species on any one trip; only in seven cases were other grains found.

The honeybee exhibits two peak periods of activity when working red clover for pollen, about 10.30 a.m. and 3 p.m. (G.M.T.). Probably the plant reaches its peaks of pollen dehiscence at these times. The honeybee probably collects pollen from red clover whenever it is available in quantity.

The visits of honeybees to red clover to collect nectar and pollen are chiefly determined by the height of the nectar in the corolla tubes.

When the nectar concentration was lower than that of other honey plants, or the nectar not high enough in the corolla tube, bumble-bees were usually more abundant on red clover than honeybees, except at the peak periods of pollen collection. When conditions were right for nectar collection five or six times as many honeybees as bumble-bees were often present. Few insects other than honeybees and bumble-bees were found working red clover.

Dunham (1939*a*) found that honeybees performed approximately 82% of the cross-pollination of red clover, bumble-bees 15% and the hymenopterous, lepidopterous and dipterous pollinating insects 3%. No comparable data collected in this country are

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available, but observations indicate that honeybees are responsible, for an important part in the cross-pollination of the seed crop.

I wish to take this opportunity of thanking Dr C. B. Williams and my colleagues in the Bee Research Laboratory for their interest and help in the investigation.

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16. THE BIOCHEMISTRY OF SILICIC ACID
9. ISOLATION AND IDENTIFICATION OF MINERALS IN
LUNG RESIDUES AND AIR-BORNE DUSTS
FROM COAL MINES

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(Received 30 December 1940)

THE aetiology of the occupational lung affections described as silicosis, anthracosis or more generally as pneumoconiosis is incompletely understood and a close comparison between the composition of the inhaled dusts and the minerals found in the affected lungs may be a useful preliminary to further knowledge. As most of the mineral particles in the lungs are below 5μ in diameter, identification with the petrological microscope is uncertain and X-ray diffraction apparatus becomes a necessary aid to the microscope. The qualitative knowledge of the minerals present, obtained from X-ray diffraction data, can be implemented by calculation of the minerals based on chemical or spectroscopic analysis.

It is intended in the present communication briefly to describe the methods used for the isolation of minerals from the lung tissue and for the X-ray diffraction analysis of these residues. The removal of coal and of acid-soluble material from air-borne dust from coal mines is also described, and data for a lung residue and an air-borne dust are presented. The chief minerals so far identified in this work are quartz, a member of the mica group and a member of the kaolin group.

Preparation of lung residues

Several methods have been proposed for preparing lung residues for X-ray diffraction work, and in some cases the same residues have been used for microscopic, chemical or spectroscopic analysis.

(1) *Simple drying of formalin-hardened lung tissue.* This method has been used by Sweany *et al.* [1938]: it has the advantage that the minerals are less altered than in any other method, but they are very much diluted by organic tissue and the resulting diagrams are poor (cf. Pl. 1A).

(2) *Destruction of organic material by digestion with acid.* HNO_3 or other acid mixtures have been employed, usually followed by ignition at $600\text{--}700^\circ$ [Jones, 1933; Kahane & Antoine, 1936; Jephcott *et al.* 1938; Burke & Kerr, 1938]. It gives a residue well suited for X-ray diffraction analysis, but it is always possible that minerals have been destroyed or altered by the heat treatment or by the acid attack. Burke & Kerr give evidence that even such a stable mineral as sericite shows after the treatment an X-ray diagram in which the lines are displaced against the lines of the untreated mineral. Any kaolinite present in the lung residues examined by the above investigators would have been destroyed by the heat treatment alone, as this mineral is destroyed by heating above 510° .

(3) *Destruction of organic material by enzymes.* Hicks *et al.* [1937] used this method. It is, however, slow, only part of the tissue can be removed and it is not easily applied to formalin-hardened tissue.

(4) *Removal of organic material by H_2O_2 .* This method, described by Sundius *et al.* [1936] and recently used by Gärtner [1939], is in our opinion the best available, as it avoids the action of high temperatures and strong acids. As modified for the purposes of the present investigation, there are three stages in the treatment:

1. Removal of fatty material from powdered oven-dry lung with acetone.
2. Digestion with H_2O_2 .
3. Extraction of the residue by acid or buffer solution.

The object of the third stage is to remove the endogenous inorganic constituents of the lung, mainly Ca, Fe, K, Na and P, as completely as possible with minimum alteration of the minerals, which form the dust in the lung. It may in some cases be impossible to attain both objectives quantitatively, but a fairly good separation can be made with repeated extraction with hot disodium hydrogen citrate at pH 6, or by brief treatment with cold *N* HCl. We have found that even prolonged treatment with *N* HCl or 2*N* HCl does not affect the appearance of X-ray diffraction diagrams of lung residues or mine dusts, as far as the diffraction lines of quartz or silicates of the kaolin and mica groups are concerned. That some small destruction of the quartz or more likely of the layer lattice silicates does take place is shown by the fact that SiO_2 is found in solution in the HCl extract, but this represents only a few per cent of the total silica in the dust or lung residue and would therefore not be reflected in X-ray diagrams with our present technique. Some results illustrating the extent of decomposition of mineral dusts by cold *N* HCl are shown in Table 1. With lung residues, decomposition and losses with cold *N* HCl and with hot disodium hydrogen citrate solution (pH 6) were slight. The amounts of SiO_2 in solution in the combined extraction and wash fluids have never exceeded a few mg., and much of this is derived from the amorphous silica of the lung tissue. The amounts of dissolved SiO_2 found in the cold HCl extracts have not been greater than those in the hot citrate extracts.

Table 1. *Treatment of powdered minerals and of air-borne dusts from coal mines with HCl*

1 g. powder shaken with 50 ml. *N* HCl at room temperature for 5 hr.

	mg. SiO_2 dissolved	Decomposition %*
Powdered minerals:		
Quartz	0.33	0.03
Kaolin	0.34	0.07
Mica sericite (Ohio)	1.81	0.45
Mica illite (S. Wales)	4.65	1.0
Air-borne dusts:		
2H2 (7.2% SiO_2)	1.26	1.8
2M1 (6.9% SiO_2)	1.22	1.8
1S1 (2.9% SiO_2)	0.48	1.7
1U1 (6.4% SiO_2)	0.55	0.9

* $100 \times \text{Dissolved } SiO_2 / \text{Total } SiO_2 \text{ per 1 g. powder.}$

The lung residues obtained by the H_2O_2 method may, however, contain large amounts of carbon (up to 80 %), and the same applies to dusts collected in coal mines. Such carbon can be removed by prolonged heating of the samples to

380° in an electric muffle. This temperature is sufficiently high to oxidize the carbon in 40–80 hr. and at the same time low enough to avoid destruction of the kaolinite lattice. Pyrites and other sulphides and hydroxides which may be present in air-borne dusts would be oxidized or dehydrated by this treatment, and they should either be centrifuged out of the dust by means of heavy liquids before heating, or identified by the X-ray diagrams of the corresponding oxides, which would be present in the heated but not in the unheated material.

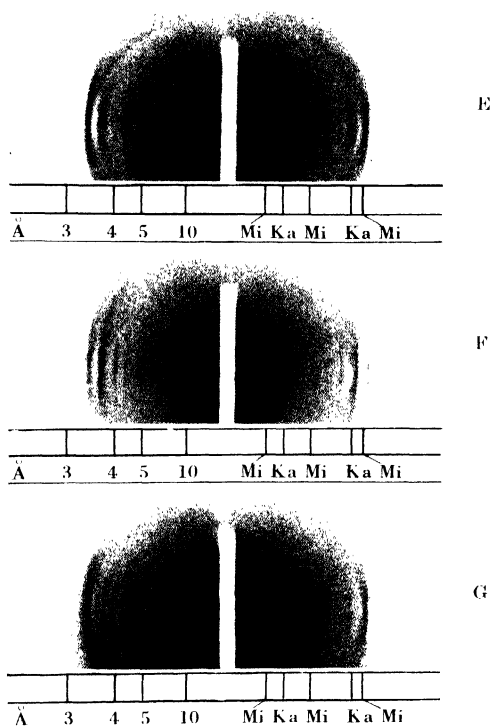
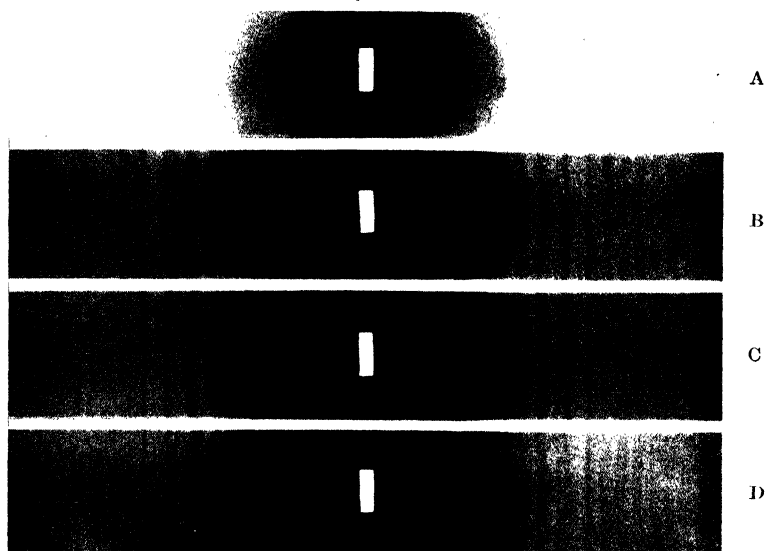
X-ray diffraction methods

In addition to taking X-ray powder diagrams in a cylindrical camera, we have used the aggregate method which has recently been described [Nagelschmidt, 1939] in its application to soil colloids. This method is specially suitable for the identification of layer lattice silicates such as mica, kaolinite, chlorite and montmorillonite in mixtures with each other. Ordered aggregates are made by slow evaporation of a suspension of the sample to be examined, and the aggregates are mounted similarly to single crystals in an X-ray spectrometer. The particles in the aggregate are oriented with their cleavage planes parallel to the plane of sedimentation and a set of good X-ray reflexions from the basal planes is therefore obtained. These basal reflexions are the main X-ray diffraction characteristics for the different layer lattice silicates. Kaolinite has strong basal reflexions at 7.14 and 3.57 Å., and mica has strong reflexions at 10 and 3.34 Å. and a weaker one at 5 Å. When aggregates of mixtures of these two minerals are heated to 510°, only the mica reflexions remain; the kaolinite reflexions are absent, as kaolinite is destroyed by heating to 500°. Chlorite shows strong reflexions at 14.1, 7.05, 4.70 and 3.52 Å. and these reflexions are still present in aggregates heated to 510°. The aggregate method is used in addition to, but not as a substitute for, the powder method. Powder and aggregate diagrams of a lung residue and a mine dust are shown in Pl. 1.

X-ray diffraction diagrams of lung residues and mine dusts indicate qualitatively the presence of different minerals if these minerals occur in sufficient amounts. Whether and, if so, how far both the powder and aggregate methods can be developed quantitatively is at present unknown. The quantitative estimation of quartz in mine dusts has been described by Clark & Reynolds [1936], but as it is based on the intensity of the 3.34 Å. reflexion of quartz it cannot be used in the presence of mica, which gives a strong reflexion for the same spacing. The method can, however, be modified, so as to be applicable to mixtures containing quartz and mica.

Preparation details

The lung is dried at 105° and reduced to a fine powder. 20 g. of the dried substance are extracted in a Soxhlet overnight with acetone to remove fatty material. The extraction thimble with its contents is dried in an oven; the contents of the thimble are knocked into a 2 l. pyrex beaker; 30 % H_2O_2 is added to a depth of about 1 in., and the mixture is carefully warmed on a water bath and stirred with a glass rod. If the froth tends to rise to the rim of the beaker, it can be whipped down with the stirring rod and if necessary octyl alcohol is added. As the frothing subsides, and as the liquid evaporates, more H_2O_2 is added. The depth of the liquid in the beaker should not exceed about 1 in., otherwise the mixture will tend to froth over. The additions of H_2O_2 , with subsequent evaporations almost to dryness, should be continued until the additions no longer cause frothing. At this point the destruction of the lung tissue seems to be complete and any black material remaining is probably coal dust.



X-Ray diffraction diagrams of a lung residue and a mine dust. A-D, Powder diagrams; E-G, aggregate diagrams. A, Oven-dry lung tissue; B, lung residue, 380° ash; C, mine dust, 380° ash after acid washing; D, mine dust, 380° ash before acid washing; E, lung residue, 380° ash; F, mine dust, 380° ash; G, same as F after heating to 520° . For reproduction, intensities in the centres of A-D have been reduced by partial shading.

The whole process, in our experience, takes about a week. The contents are brought nearly to dryness each evening. Each morning, and as often during the day as the mixture comes to dryness, more H_2O_2 is added.

The final residue consists of the inorganic salts from the lung tissue plus the dust. The former are brought into solution by extracting the mixture with acid. We have used *N* HCl with 15 min. vigorous shaking at room temperature, and hot *M*/10 citrate buffer of pH 6 with continuous stirring for 1 hr. On allowing the mixture to settle, the supernatant liquid is seen to contain much of the iron in solution. Separation of the solid from the liquid can be accomplished by gravity filtration through a fine paper (a hardened filter paper seems to retain the dust particles), or by prolonged centrifuging. We have found it convenient to transfer the residue, with washing, to 250 ml. centrifuge bottles, and to use 200 ml. portions of liquid for each extraction. Each separation is carried out by centrifuging for 1 hr. at 3500 r.p.m.

The residues are re-extracted several times to remove all salts. Iron is the most difficult of the tissue constituents to eliminate from the residue, and it is usually necessary to extract the centrifuged deposits three or four times with cold HCl or hot citrate buffer. The presence of iron in the extraction fluids may be tested for by treating a sample with acetic acid, sodium acetate and ferrocyanide.

The filtrate or supernatant fluid should be checked for the absence of dust particles by microscopic examination, and by colorimetric analysis for total SiO_2 [King, 1939]. The presence of appreciable numbers of dust particles will indicate the necessity for more prolonged centrifuging. With the residues we have handled, 1 hr. spinning appears to have been sufficient. The presence of non-particulate SiO_2 , i.e. dissolved SiO_2 , in any quantity indicates a decomposition of the siliceous matter by the extraction fluids. The residue is finally washed once with water and once with alcohol and is dried in an air oven.

Results

As an example, we give full details for one lung residue and one mine dust. The lung was kindly supplied by Dr P. D'Arcy Hart and the mine dust was collected by Dr P. F. Holt, working with Prof. H. V. A. Briscoe. The occupational history of the case from which the lung residue was taken will be given elsewhere, in conjunction with the results obtained in the study of a large group of workers.

Lung residue. The lung residue prepared in this way lost 12% of the dry weight on prolonged heating to 380° . Powder and aggregate X-ray diffraction diagrams of the 380° ash taken with unfiltered iron K radiation are shown in Pl. I, B and E. A powder diagram of the untreated oven-dried powdered lung tissue is shown in Pl. I, A. Table 2 gives the interplanar spacings and intensities of diffraction lines of the 380° ash diagram, and the interpretation in terms of quartz, mica and kaolin. There are many diffraction lines of quartz which do not coincide with other lines in the diagram, but only two lines, the basal reflexions, which can be ascribed to kaolin alone. It is not possible to distinguish under these conditions which member of the kaolin group it is; it can only be said that a member of the group is present. The kaolin group contains the minerals kaolinite, nacrite, dickite, anaxite and halloysite. No other mineral is known to give strong basal reflexions at 7.1 and 3.55 Å. spacings, which disappear if the sample is heated to 510° . For the mica it is likewise not possible to determine which member of the mica group is present. The diagram does not seem to correspond to well-crystallized muscovite or hydromuscovite, but rather to the

type of mica found in shales and soil colloids. The term 'illite' [Grim *et al.* 1937] has been proposed for mica of this type, but samples described as sericite or secondary muscovite may refer to similar material.

Table 2. *X-ray data for lung residue, 380° ash*

d = interplanar spacings, Int. = intensities of diffraction lines, v.s. = very strong, s. = strong, m. = medium, w. = weak, v.w. = very weak. Q. = quartz, Mi. = a member of the mica group, Ka. = a member of the kaolin group.

} denotes edges of a band or two lines of different intensity which are not clearly separated.

No.	d	Int.	Mineral	No.	d	Int.	Mineral
1	10.1	s.	Mi.	13	1.815	s.	Q.
2	7.1	s.	Ka.	14	1.675}	m.	Mi., Ka., Q.
3	5.0	w.	Mi.	15	1.644}		
4	4.48}	s.	Mi., Ka.	16	1.539}	m.	Q.
5	4.23}	m.	Q.	17	1.508}		
6	3.56	m.	Ka.	18	1.488}	s.	Mi., Ka.
7	3.35}	v.s.	Q., Mi.	19	1.382}	s.	Q.
	3.31}			20	1.372}		
8	2.58}	s.	Mi., Ka.	21	1.294}	w.	Mi., Ka.
	2.54}			22	1.280}		
9	2.46	v.w.	Mi., Q.	23	1.200	m.	Q.
10	2.45}	v.w.	Ka., Q., Mi.	24	1.180	m.	Q.
	2.18}			25	1.151	w.	Q.
11	2.131	m.	Q., Mi.	26	1.076	s.	Q.
12	2.003}	m.	Mi., Ka., Q.				
	1.972}						

Table 3 gives the chemical analysis of the lung residue carried out on the 380° ash. The water lost below 105° shown in Table 3 has been reabsorbed from the air by the ash. It will be seen that SiO_2 , Al_2O_3 , K_2O , and water above 105°, account for 94 % of the residue, and these four are the main chemical constituents of a mixture of quartz, muscovite and kaolin. It is possible to calculate the relative amounts of the three minerals in the lung residue if it is assumed that all the SiO_2 , Al_2O_3 and K_2O are present in these minerals, and if the composition of the muscovite and of the kaolin are known. Such calculations with kaolin and muscovite of theoretical composition give for the lung residue: muscovite 26 %, kaolin 46 % and quartz 24 %. The chemical composition of

Table 3. *Chemical analysis of lung residue ashed at 380°*

Analyst: Chemical Laboratories, London

SiO_2	56.9
Al_2O_3	28.3
TiO_2	1.4
Fe_2O_3	1.5
MgO	0.7
CaO	0.5
Na_2O	0.8
K_2O	3.1
H_2O , above 105°	5.4
H_2O , below 105°	1.1
P_2O_5	0.1
	99.8

mica may vary within wide limits, and the mica found in shales and soil colloids usually contains only 4-6 % K_2O , as against muscovite with 11.8 % K_2O . Calculation of the minerals in the lung residue with a mica containing 5 % K_2O , 48 % SiO_2 and 32 % Al_2O_3 , and kaolin of theoretical composition, gives for the

residue a mixture of 62% mica, 21% kaolin and 17% quartz. The X-ray diffraction data indicate only that all three minerals occur as main constituents. Their true relative proportions probably lie between the two sets of percentages given above. If special interest is centred on SiO_2 , it will be seen that less than half of the total SiO_2 shown in the analysis, or less than a quarter of the mineral residue, occurs as quartz.

Microscopic inspection of the 380° ash of the lung residue shows that nearly all particles are well below 2μ in diameter and it does not seem possible to distinguish the different constituents quantitatively by their refractive index or other optical characteristics with microscopic petrological methods.

Mine dust. The untreated mine dust gave a poor X-ray diffraction diagram. After heating the material to constant weight at 380° a residue consisting of 15.1% of the original dust was obtained. X-ray powder and aggregate diagrams of this residue are shown in Pl. 1, D and F, and a diagram of the same residue after a short treatment with N HCl in Pl. 1, C. Pl. 1, G shows an aggregate diagram, taken with the same specimen as Pl. 1, F, after heating it to 540° for 24 hr. The kaolin lines are absent. Table 4 gives the evaluation of the diagram

Table 4. *X-Ray data for mine dust, 380° ash, after treatment with N HCl*

d = interplanar spacings, Int. = intensities of diffraction lines, v.s. = very strong, s. = strong, m. = medium, w. = weak, v.w. = very weak, Q. = quartz, Mi. = a member of the mica group, Ka. = a member of the kaolin group.

} denotes edges of a band or two lines of different intensity which are not clearly separated.

No.	d	Int.	Mineral	No.	d	Int.	Mineral
1	10.2	m.	Mi.	13	1.540	m.	Q.
2	7.1	v.s.	Ka.	14	1.508	m.	Mi.
3	4.48	v.s.	Ka., Mi.	15	1.493		
4	3.55	s.	Ka.		1.484	s.	Ka.
5	3.35	v.s.	Q., Mi.	16	1.380	s.	Q.
	3.31				1.370		
6	2.58	s.	Ka., Mi.	17	1.318	w.	?
7	2.55	v.w.	Mi., Q.	18	1.286	w.	Ka., Mi.
8	2.37			19	1.255	w.	Q.
	2.33	m.	Ka.	20	1.236	v.w.	Ka., Mi.
9	2.131	w.	Q., Mi.		1.225		
10	1.998	m.	Ka., Mi., Q.	21	1.200	m.	Q.
	1.980			22	1.180	m.	Q.
11	1.816	m.	Q.	23	1.151	w.	Q.
12	1.671	s.	Ka., Mi., Q.		1.076	m.	Q.
	1.642						

shown in Pl. 1, C. It is again only possible to state that quartz and members of the kaolin and mica groups are present. The presence of a line at 14.3 \AA . on aggregate diagrams may possibly indicate minor amounts of chlorite. The diagram before the N HCl treatment shows a few additional diffraction lines indicating dolomite or ankerite. Dolomite is a calcium-magnesium carbonate and ankerite a calcium-magnesium-iron carbonate.

Table 5 gives the chemical analysis of the 380° ash of the mine dust. SiO_2 , Al_2O_3 , K_2O and water above 105° account for 86% of the ash and most of the remaining Fe, Ca and Mg is present as carbonate, sulphate or oxide, although a proportion of these may form chlorite with some of the SiO_2 and Al_2O_3 . The mineral content has been calculated ignoring chlorite and assuming all SiO_2 , Al_2O_3 and K_2O to be present as quartz, kaolin or muscovite of theoretical composition. The mine dust ash contains according to this calculation 14% muscovite, 66% kaolin and 11% quartz. Assuming a mica containing 5% K_2O ,

Table 5. *Chemical analysis of mine dust ashed at 380°*

Analyst: Geochemical Laboratories, London

SiO ₂	47.9
Al ₂ O ₃	31.6
TiO ₂	0.8
Fe ₂ O ₃	3.9
MgO	1.4
CaO	3.1
Na ₂ O	1.0
K ₂ O	1.6
H ₂ O, above 105°	4.6
H ₂ O, below 105°	0.7
CO ₂	0.8
SO ₂	2.2
S	0.1
	<hr/> 99.7

48 % SiO₂ and 32 % Al₂O₃ the mine dust would contain: 32 % mica, 54 % kaolin, 8 % quartz. The water above 105° shown in Table 5 is too low to satisfy the amount of lattice hydroxyl present in kaolin of theoretical composition for either of these mixtures, but many kaolin samples contain less than the theoretical amount of water and part of the lattice hydroxyl may have been removed by the initial prolonged heating to 380°.

Free SiO₂ (quartz) [Shaw, 1934] amounted to 12 % of the mine dust ash, in fair agreement with the estimate of 11 % from the calculation with muscovite and kaolin of theoretical composition. We are grateful to Mr A. Shaw for carrying out this determination.

SUMMARY

The isolation of mineral residues from lungs by the H₂O₂ method and the removal of coal from such residues and from air-borne dusts from coal mines by heating to 380° are described. X-ray diffraction methods are used for the identification of minerals in the residues and full data, including chemical analyses, presented for a lung residue and a mine dust. Quartz, a member of the kaolin group and a member of the mica group are the main constituents found in the lung residue and in the air-borne dust.

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DERMATITIS CAUSED BY INSECTICIDAL PYRE-
THRUM FLOWERS (*CHRYSANTHEMUM*
CINERARIIFOLIUM).

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INTRODUCTION.

THE use of the powdered flowers and of extracts of *C. cinerariifolium* in the control of insect pests has increased considerably in recent years, and the plant now ranks as one of our most important insecticides. Pyrethrum has played an important part in the development, and forms a major constituent, of the modern domestic fly-spray. In addition to its use in agriculture and horticulture, it has provided a satisfactory means of controlling certain insect pests of stored food products. Its value as an insecticide is by no means fully worked out.

Japan, Kenya and Yugoslavia are the chief centres of production. Cultivation of the plant was commenced in Kenya some ten years ago, and the country now exports about two thousand tons of dried flowers per annum. There the plant flowers over ten months of the year, permitting a steady supply of flowers of excellent quality.

The insecticidal principles consist of the pyrethrins I and II. These are esters of a cyclic ketone, pyrethrolone, with two acids, one a monocarboxylic acid, and the second the methyl ester of a dicarboxylic acid. The pyrethrins may occur in quantities up to

two per cent. of the air-dried flowers. Pyrethrin I is one of the most potent insecticides known, the median lethal concentration for aphids being about 10 mg. per litre of spray solution.

The pyrethrins show a well-marked toxicity for many types of cold-blooded animals, and the possibility of their use in the control of pests of man and the higher animals becomes a matter of some interest. Chevalier (1930) and Gaudin and his co-workers (1932) have recommended pyrethrum for the treatment of *Trichocephalus*, *Taenia*, and *Ascaris* in humans. Sweitzer and Tedder (1935) found an ointment containing 0.75% of the pyrethrins to be of value in the control of scabies. Dermatitis was produced only in rare instances, and there was no evidence of toxic absorption. Pyrethrum has been recommended for the control of the human body louse, a kerosene extract of pyrethrum emulsified with soap being stated to kill the eggs as well as the adults. Pyrethrum ointment and oil extracts have also been suggested for use against the harvest mite.

The belief that pyrethrum is non-toxic to man appears to be well founded. Research workers have handled the pure pyrethrins, and doses have been taken orally without ill effect. Workmen have been employed daily for years in grinding the flowers or in contact with extracts without showing toxic symptoms. It is well known, however, that some workers are affected with dermatitis following contact with the flowers or extracts, while in others disturbances in the respiratory tract may result. Feinberg (1934) is of the opinion that pyrethrum powder contains, in addition to the insecticidal esters, an oleo-resin which is probably responsible for the dermatitis, and a specific allergen which causes the respiratory troubles. Many cases of pyrethrum allergy have indeed been reported. There have been a considerable number of cases of dermatitis and sensitization among both Europeans and Africans handling pyrethrum in Kenya. In some cases, planters have been obliged to leave the vicinity of pyrethrum fields during the harvesting period. Tonking (1936) states that two or three years may be required for susceptibility to appear and this has been the experience of one of us. Sequeira (1936), in an account of the incidence of pyrethrum dermatitis there, clearly differentiates between the symptoms which are due to irritation and those, much rarer, which are allergic.

It is generally recognized that, in commerce, the flowers are more liable to cause dermatitis than are extracts, and that susceptibility is increased during summer or periods of excessive perspiration. McCord, Kilker, and Minster (1921) conclude that the risk of dermatitis chiefly occurs during operations of weighing and grinding of the crude drug, and that the irritant responsible is not necessarily insecticidal. They describe clinical manifestations of a skin-disease confined to a department handling pyrethrum. These consist of a mild erythema with much itching, a vesicular dermatitis, and papules in moist areas. Alleviation occurs within a few days after removal from the dust. A well-known firm in this country who have ground pyrethrum for many years state that they have met with some half-dozen minor cases of sensitiveness, and one man who was seriously ill after exposure to pyrethrum powder. They have the impression that dermatitis is much less likely to result from the handling of extracts. In factories, risk of dermatitis may be materially reduced by the use, as far as possible, of dust-proof machinery and automatic packing and weighing.

One of us (J. T. M.) has for some years been susceptible to pyrethrum dermatitis. He has been actively engaged with the chemistry and biology of this plant for ten years. During the first two or three years no ill-effect was noticed, and then susceptibility appeared suddenly during harvesting operations in hot weather. The symptoms were intense itching and sensitiveness of the hands and face, with swelling of the eyelids. In all subsequent work with the flowers, either in the fresh condition or in the air-dried state, all exposed parts of the body have had to be smeared with vaseline in order to avoid ill-effect. Sensitization is so acute that, without this, a field of insecticidal pyrethrum may be detected at an appreciable distance. The presence of an open tin of air-dried flowers in the laboratory is quickly recognized. A tingling of the face results, the chin, area around the nose, and the eyelids being particularly sensitive. Susceptibility is increased during the hot weather, and the effect disappears soon after removal from the source of irritation. With one exception, which will be mentioned later, no ill-effect has been observed during work with extracts. It has been observed by us that fair people are likely to be more susceptible than dark.

The present work was undertaken to ascertain the causal agent of the dermatitis. We considered it important to determine whether

or no the pyrethrins are responsible. If the active agent is something other than the pyrethrins, its removal from extracts might be feasible, permitting the preparation of insecticidal products devoid of dermatitis-producing properties.

The chemical work has been carried out by J. T. Martin, the flowers being handled by a laboratory assistant. K. H. C. Hester has been responsible for the clinical control of the investigation and the description of the reactions. In view of his susceptibility, J. T. Martin has been used as the test-subject throughout the work.

EXPERIMENTAL.

Technique.

The product to be tested was evenly spread over the "fluffy" side of a one-inch square of flannel-vesting material. This was then fixed to the skin by strips of adhesive strapping and allowed to stay for a minimum period of twenty-four hours. In the early work the period allowed was 48 hours; but with concentrates tested later the patches were removed soon after 24 hours in order to lessen the risk of toxic absorption. The patches were usually applied at 7.0 p.m., the sites used being the anterior aspect of the forearms, the thighs, and the trunk in the region of the right iliac crest. Patches were not applied unless the site, if previously used, was completely healed and the skin free from blemish. When resinous material was examined, the test area was washed with alcohol after the removal of the patch, and observations were made after an interval of at least one hour. Tests were made during the summer months in order to obtain a maximum sensitivity.

Six criteria were used in judging the intensity of a reaction. These were: (a) the degree of erythema, (b) the uniformity of the erythema over the patch site, (c) the amount of oedema, (d) the extent of vesiculation, (e) the intensity of irritation, (f) the duration of the dermatitis. With intense reactions, the outline of the patch was clearly defined, there was marked oedema and vesiculation, intense irritation, and persistence of the inflammation. Each reaction was judged and accorded a sign, ranging from a negative sign for complete absence of effect to four plus signs for an intense reaction (see Table I). The assessment of the reaction was made

at the time of removal of the patch and was independent of the period of application.

The powdered flowers and various fractions of the extract were submitted to analysis for pyrethrin content. This was carried out by the method of Seil (1934) with modifications. Free acids were removed from the petroleum ether extracts with dilute alkali, and saponification was effected in 0.5 N methyl alcoholic potash solution. The methyl alcohol was then removed at a low temperature under reduced pressure. The filtrate from the barium precipitation was neutralized to litmus with normal sulphuric acid, and 1-2 ml. were added in excess prior to distillation of the mono-carboxylic acid.

Tests with Flowers.

Degree of susceptibility.—It was first necessary to determine that J. T. Martin was still susceptible to pyrethrum dermatitis and the degree of effect likely to be produced. Patch tests were therefore made on 15 June 1940 upon the anterior aspect of the left forearm with powdered pyrethrum flowers. Two patches were applied, one consisting of the air-dried flowers and the other of an equivalent quantity of the powder moistened with water.

The flowers had been grown on an experimental plot in Harpenden, and harvested in 1938 and 1939. The plants used were derived from one parent plant (our ref. no. F.11). On analysis soon after harvesting, the 1938 crop showed 0.91% of pyrethrin I and 0.37% of pyrethrin II, and the 1939 crop 0.92% of I and 0.34% of II. The values for the mixed flowers in June 1940 were 0.85% of pyrethrin I and 0.31% of pyrethrin II.

The patches were removed after 48 hours. Irritation was first felt after 24 hours, and increased in intensity up to the time the patches were removed. The reactions were as follows:

(a) *Air-dried flowers.*—There was a moderate erythema, but no swelling. The erythema was not uniform, but was more intense where subjected to pressure by the strapping. The surface was punctate, suggesting the impending formation of vesicles.

(b) *Moistened flowers.*—The erythema was more intense and was uniform, and there were many small vesicles and some swelling.

A control with vesting and bandage alone gave no reaction.

After the patches were removed, the area subjected to the moistened flowers was more irritable than that covered by the dry flowers, and the effect persisted longer. By 25 June the reactions had almost completely subsided, with the skin beginning to peel. The outlines of the patches were still distinctly visible, and there was occasional irritation. It was clear that J. T. Martin was still susceptible to pyrethrum dermatitis, and also that moistened flowers gave a more vigorous reaction than dry flowers.

Tests with fractions of the flower head.—Pollen was dusted from a large number of flowers, while other flower-heads were dissected into their

constituent parts, ray florets, disc florets, ovaries, and the residue consisting of receptacles and involucre. Control patch tests with the pollen and air-dried ground flowers were carried out on the anterior aspect of the right forearm on 18 June, while the dissected fractions of the flowers, sliced into small pieces, were tested on the right thigh, with moistened ground flowers as control on the left thigh, on 25 June. The pollen and control patches were removed after 48 hours and the others after 42 hours. The reactions were as follows:

(c) *Pollen*.—A definite, but only slight, erythema, which rapidly faded.

(d) *Receptacles and involucre*.—Moderate erythema; area of test slightly swollen. No vesicles.

(e) *Petals*.—Slight erythema; no swelling.

(f) *Ovaries*.—Moderate erythema; area slightly raised.

(g) *Disc florets*.—Slight erythema; no swelling.

The reaction of (d) and (f) were of approximately equal intensity, which was greater than that of reactions (e) and (g), which were also about equal.

(h) *Moistened ground flowers* (control).—Fairly intense erythema, edge of patch well defined, and whole of test area raised.

It was clear from these tests that the pollen could not be regarded as carrying the agent responsible for dermatitis, and that, furthermore, this appeared to be concentrated in the lower portion of the flower head, i. e. in the ovaries, receptacles and involucre. It is of interest to note that while the pyrethrins are known to be concentrated in the ovaries, the receptacles and involucre contain only small amounts of the insecticidal principles (Martin and Tattersfield, 1934), providing evidence that the causal agent of the dermatitis is likely to be something other than the pyrethrins.

Tests with Extracts of the Flowers.

Pyrethrum extract in heavy oil.—Tests were first carried out, on 18 June, with a commercial preparation of pyrethrum, consisting of an extract of the flowers made up in a highly refined heavy mineral oil (Shell No. 24210) similar to medicinal paraffin. The oil solution contained about 1% of total pyrethrins.

The pyrethrum preparation was sprayed, in an even film, on to squares of flannel vesting. The deposit used was of the order of 2 mg. per cm.² Patches were then applied to the anterior aspect of the right forearm and to the trunk just above the right iliac crest and behind the anterior superior iliac spine. Control tests were made with oil alone on the left forearm; and with air-dried ground flowers on the right forearm and on the trunk just behind the test patch. The patches were removed after 48 hours. The reactions were as follows:

(i) *Pyrethrum extract in heavy oil* (right forearm).—Faint mottled erythema covering the area of the patch, with one small vesicle. No irritation.

(j) *Ground flowers* (right forearm, control).—Vigorous reaction. The whole area was red and slightly raised. Around the test area there was a flush, $\frac{1}{2}$ to $\frac{3}{4}$ in. wide. Intense irritation 36 hours after application.

(k) *Pyrethrum extract in heavy oil* (trunk).—Faint, irregularly distributed erythema, more marked at the edges of the test area. No irritation.

(l) *Ground flowers* (trunk, control).—Marked redness and swelling of the skin with a spreading flush. Intense irritation after 36 hours.

The pyrethrum preparation in oil gave a definite though faint positive reaction under the stringent conditions of the test. The reaction was rather more intense than that given by the pollen tested simultaneously, and persisted longer. On 26 June the outlines of the patches could still be clearly seen, with occasional slight irritation from the pyrethrum powder test areas.

Ether and petroleum ether extracts of the flowers.—An attempt was next made to determine the distribution of the causal agent between ether and petroleum ether extracts. The air-dried flowers (50 g.) were percolated with ether for seven hours, and dried in a vacuum desiccator. The ether was distilled, and the resin dried under reduced pressure at a low temperature. It was then extracted four times by refluxing with low boiling petroleum ether, and the petroleum ether-soluble fraction of the resin was recovered.

Patches were applied in the evening of 25 June, using the ether-extracted flowers (moistened with water), and the fractions of the resin soluble and insoluble in petroleum ether. The site used was the left thigh, test (h) of ground flowers serving as control. The patches were removed after 42 hours, when the reactions were as follows:

(m) *Ether extracted flowers* (moistened with water).—Faint, ill-defined erythema. No swelling.

(n) *Petroleum-ether-insoluble fraction of the resin.*—Brisk reaction, with well-defined raised edge. Vesicles.

(o) *Petroleum-ether-soluble fraction of the resin.*—Less intense redness than in test (n), but edge of the area was well defined and raised.

The control test (h) was not distinguishable from test (o). Irritation was first felt 12 hours after application of the patches and became intense by the morning of 27 June. At this time irritation of the face, particularly round the lower jaw and mouth, and a slight puffiness and redness under the right eye were noticed. A general constitutional reaction ensued, which is described later.

The skin-lesions persisted, with varying intensities of irritation, until 15 July, when the outlines of the patches were still faintly visible. On 12 July there was intense irritation from patch test (n). The area had become red, and there was exudation of fluid. On 15 July the area showed scaling with a tendency to crack and exude fluid—a characteristic eczematous reaction.

It is clear that ether almost completely extracts the active agent from the flowers. A surprising effect was the intensity of the reaction given by the oleo-resin after it had been extracted by petroleum ether. It may be assumed that the pyrethrins, readily

soluble in petroleum ether, would to a large extent pass into the petroleum ether extract, providing further evidence that it is unlikely that they can be responsible for the dermatitis.

Colourless extract of pyrethrum.—Petroleum ether is normally used as solvent for the extraction of pyrethrum for analysis, separating the bulk of the pyrethrins with a minimum of extraneous matter. The extract, however, contains an appreciable quantity of fatty material, free acids, and pigments. Petroleum ether extraction of the flowers used in this work gave a resin containing approximately 30% of total pyrethrins. It has been shown by Martin and Potter (1937) that, if the powdered flowers are intimately mixed with decolorizing charcoal and then extracted with petroleum ether in a Soxhlet apparatus, a colourless extract results. This contains a higher content of the pyrethrins (about 45%) and very little free acids. In order to determine whether the causal agent of the dermatitis was retained on the charcoal or extracted by the solvent, a patch test was made on 15 July, using a colourless extract prepared from the flowers previously used, on the lower part of the anterior aspect of the right forearm. The patch was removed after 48 hours.

(p) *Colourless extract of flowers.*—This gave an intense reaction. The square of skin was yellowish and exhibited well-marked capillary pulsation, flushing at each systolic pulse-beat. It was well raised and the surface was dotted with numerous small vesicles. The skin around showed a pink flush for a distance of 1 in. There was more oedema of the skin than in any previous reaction, and intense irritation.

By 22 July the test area was dull red, still slightly raised, and finely desquamating. It began to peel on 25 July.

In view of the intense reaction it was decided to concentrate future work upon the component fractions of the colourless extract. The major constituents are the pyrethrins and fatty material. Much smaller quantities of the essential oil originally in the flowers and of crystalline constituents, such as chrysanthin, are also present. It was decided first to test the fat fractions.

The colourless oil was redissolved in petroleum ether and washed with dilute alkali to remove the small amount of free acids, after which the solvent was again removed. The fats were then separated by the following procedure, based upon the work of LaForge and Haller (1935). The colourless extract was dissolved, with warming, in glacial acetic acid. An equivalent amount of acetic acid, plus 20% of its volume of water, was added with swirling and the solution kept at 0° C. The fatty material precipitated was filtered, washed with cold acetic acid containing 10% of water, copiously with water, and allowed to dry at room temperature.

A patch test extending over 48 hours was made on 22 July, on the lower part of the anterior aspect of the left forearm. The reaction was as follows:

(q) *Fatty material separated from a colourless extract.*—Moderately intense mottled erythema. The red patches were slightly raised. No vesicles, or surrounding flush. Some irritation after removing the patch. This represented a positive reaction of slight intensity.

It has been observed (Tattersfield and Martin, 1934) that if air is

bubbled through a methyl alcoholic extract of pyrethrum in sunlight, fatty material gradually separates. The nature of this product is at present indefinite. Commercial users of pyrethrum believe that old flowers are more likely to cause dermatitis than are fresh flowers, and it was thought possible that an oxidation product of the fatty constituents of the flowers might be responsible.

The flowers, admixed with charcoal, were extracted with petroleum ether, a pale yellow resin resulting. This was extracted with successive portions of methyl alcohol, with cooling and filtering of each. The resulting clear solution was aerated for three days, and the precipitate separated, washed, and dried. A patch test was carried out on 25 July on the middle of the anterior aspect of the right forearm.

(r) *Fatty material separated after aeration of a methyl alcoholic extract of pyrethrum.*—After 48 hours there was an irregular mottled erythema of slight intensity, slightly raised. No vesicles, and no irritation.

Tests (q) and (r) seemed to us effectively to dispose of the possibility that the fat fraction of the extract could be the causal agent of the dermatitis. There remained the pyrethrins and minor constituents. It was decided at this stage to attempt the preparation of a concentrate as rich as possible in the pyrethrins, other products separated to be tested as obtained.

Preparation of pyrethrin concentrate.—The flowers previously used (700 g.) were intimately mixed with 420 g. of decolorizing charcoal (old) and extracted for several days with low boiling petroleum ether. The solution obtained was pale yellow. The resin contained about 40% of pyrethrins, made up of 30% of I and 10% of II. The petroleum ether solution was concentrated to 125 ml., filtered and kept at 0° C. for a week.

A white crystalline material separated. This was removed, washed with petroleum ether, and recrystallized from absolute alcohol (test (s)). Water was added to the petroleum ether solution and free acids were removed by the gradual addition of 0.5% potash solution, with shaking. The petroleum ether solution was washed with water, dried over anhydrous sodium sulphate, and the yellow resin recovered. The resin was dissolved with gentle warming in 20 ml. of glacial acetic acid. On cooling, a precipitate resulted. This was filtered off, and washed with 10 ml. in all of acetic acid.

The yellow precipitate was dissolved in absolute alcohol, the solution strained from a small amount of insoluble yellow "liquid" resin (test (t)) and cooled in the refrigerator. Colourless needles separated, with occluded small yellow globules (test (u)). The yellow "liquid" resin solidified on standing.

To the acetic acid filtrate and washings (30 ml. in all) was added 10 ml. of acetic acid and 4 ml. of water. The solution was kept at 0° C. for 2 days. The precipitated fats were filtered off, and the clear filtrate extracted with two volumes of petroleum ether. The petroleum ether solution was washed four times with acetic acid containing 10% of water, then four times with water, dried over sodium sulphate, and the resin

recovered. This consisted of 4.2 g. of a pale yellow oil, and contained a total of 78% of pyrethrins, made up to 65% of I and 13% of II.

Further purification was effected as follows: 2 g. of the oil was dissolved in low boiling petroleum ether and was slowly run through a column of (fresh) decolorizing charcoal 3 in. by $1\frac{1}{4}$ in. in diameter. The charcoal was previously wetted with petrol ether, and so packed as to give a steady rate of flow. The charcoal was then washed six times with 25 ml. portions of petroleum ether. The filtrate yielded 0.07 g. of a slightly pungent oil. Further continuous washing of the charcoal with petroleum ether for six hours resulted in an additional 0.03 g. of oil.

The charcoal was allowed to dry at room temperature, and was then washed by continuous percolation with ether for 6 hours. The filtrate yielded 1.33 g. of a colourless oil. Further percolation with ether for 6 hours yielded an additional 0.2 g. of colourless oil.

The charcoal was again dried, remixed, and percolated with chloroform for $7\frac{1}{2}$ hours, yielding 0.25 g. of a yellow resin.

In all, 93% of the resin taken was thus accounted for. The colourless oil first extracted by the ether on analysis showed a content of 93% of total pyrethrins (duplicate analyses gave 81.7, 81.0% of I and 12.1, 11.3% of II).

A patch test was made with this pyrethrin concentrate on 9 August (test (v)).

The reactions given by the products obtained during the preparation of the pyrethrin concentrate were as follows:

(s) *Material separating from the petroleum ether extract, recrystallized from alcohol.*—The patch test was made on 8 August on the middle of the anterior aspect of the left forearm. The patch was removed after 46 hours. There was an ill-defined irregular red mottling, slight swelling, and small vesicles.

(t) *"Liquid" fraction of material insoluble in acetic acid.*—The patch was applied on 6 August to the middle of the right forearm, and was removed after 48 hours. The test area was red, uniformly raised, and dotted all over with minute vesicles.

(u) *Crystalline fraction of material insoluble in acetic acid, from alcohol solution.*—Patch test on 6 August, applied to the upper portion of the anterior aspect of the right forearm, and removed after 48 hours. This showed an erythema all over its surface with a mottled raised area (probably outlining points of contact of the crystal masses with the skin). In the raised area minute vesicles were present.

Tests (t) and (u) caused some irritation during the last 12 hours of application, and on removal of the patches this became fairly intense. Both gave moderately strong reactions, that of (u) being somewhat less than (t).

(v) *Pyrethrin concentrate (93% total pyrethrins).*—This was tested on 19 August, on the anterior aspect of the left forearm, towards its inner side and just below the elbow. The patch was removed after 27 hours to lessen the risk of toxic absorption. Tingling was felt for a few hours after application, but this did not persist. On removing the patch the test area could just be distinguished, but its outline rapidly faded. A few slight swellings were visible, and only slight irritation was felt. Twelve hours later the test area could be faintly seen as a pale pink

mottling, very ill defined. This represents a very slight positive reaction only.

There remained the possibility that the essential oil was the main cause of the dermatitis. A disturbing feature of the investigation had been the way in which all the fractions tested had given positive reactions, though of varying intensities. The only product tested which could be regarded as being uncontaminated was the crystalline material separating from the petroleum ether extract and recrystallized from alcohol (test (s)). The remaining products, with the possible exception of the pyrethrin concentrate (test (v)), smelt to varying degrees of pyrethrum flowers. Tests were therefore made with the oil separated directly from the flowers.

Preparation of the essential oil.—The ground flowers (200 g.) were soaked in water and then subjected to steam distillation. The distillate was extracted twice with ether, the solution was dried over sodium sulphate, and a pale yellow sweet-smelling oil recovered. The yield was of the order of 0.1%.

(w) *Volatile oil.*—The oil was tested on 8 August, on the anterior aspect of the upper part of left forearm. The patch was removed after 28 hours because of intense irritation and the sickening effect of the volatile oil. The whole test area was swollen, with a very distinct outline, and was evenly covered by large vesicles, some of which had broken. It was surrounded by a flush 1 in. wide. A distinct skin-lesion showed where the oil had crept between the overlying pieces of bandage. Twenty hours later the area was still slightly raised, and showed a moderately intense erythema and exudation from ruptured vesicles.

During the preparation of the oil and the wearing of the patch a tingling of the skin on the chin and around the nose was experienced.

There was a marked persistence of the reaction, the outlines of the patch still being visible on 27 August.

Separation of volatile acid from the essential oil.—It is known that acids occur in the free condition in pyrethrum flowers, some of which, including the chrysanthemum monocarboxylic acid, are volatile in steam. The acid number of the oil under examination was 87. The possibility remained that the acid fraction of the volatile oil was the causal agent of the dermatitis.

The oil was dissolved in neutral alcohol, and the solution made distinctly alkaline with 0.1 N. sodium hydroxide. Water was added, and the solution was extracted twice with ether. The ether solution was washed with water, and the neutral oil recovered.

This was submitted to a patch test on 15 August, using the lower part of the right thigh (test (x)).

The flowers, from which the volatile oil had been obtained, were dried by heating in an evaporating basin on a water bath and finally at a temperature of 35° C., until of the consistency of a thick paste. The flower material was then used for a patch test (y) on the right thigh

TABLE I.—*Summary of the Intensities of Dermatitis Reactions Produced by Pyrethrum Flowers and their Constituent Fractions.*

Test.	Material.	Site of patch.	Period of application (hours).	Intensity of reaction.
a.	Air-dry ground flowers.	Upper left forearm.	48	+++
b.	Moistened ground flowers.	Middle " "	48	+++
c.	Pollen.	Lower right " "	48	+?
d.	Receptacles and involucres.	Upper " thigh.	42	++
e.	Petals.	Middle " "	42	+
f.	Ovaries.	" " "	42	++
g.	Disc florets.	Lower " "	42	+
h.	Moistened ground flowers (control).	" left "	42	+++
i.	Pyrethrum extract in mineral oil.	Middle right forearm.	48	+
j.	Air-dry ground flowers (control).	Upper " "	48	+++
k.	Pyrethrum extract in mineral oil.	Trunk, near iliac crest.	48	!
l.	Air-dry ground flowers (control).	Trunk, posterior to (k).	48	+++
m.	Moistened ether-extracted flowers.	Upper left thigh.	42	+
n.	Petroleum ether-insoluble fraction of the resin extracted by ether.	Middle " "	42	+++
o.	Petroleum ether-soluble fraction of the resin extracted by ether.	" " "	42	+++
p.	Colourless extract of flowers.	Lower right forearm.	48	++++
q.	Fatty material separated from colourless extract.	" left "	48	++
r.	Fatty material separated after aeration of a methyl alcoholic extract.	Middle right forearm.	48	+
s.	Material separating from a petroleum-ether extract recrystallized from alcohol.	" left "	46	++
t.	" Liquid " fraction of material insoluble in acetic acid.	" right "	48	+++
u.	Crystalline fraction of material insoluble in acetic acid, from alcohol solution.	Upper " "	48	++
v.	Pyrethrin concentrate (93 per cent. pyrethrins).	" left "	27	+
w.	Volatile oil.	" " "	28	++++
x.	Volatile oil, free from acids.	Lower right thigh.	28	++
y.	Moist steam-distilled flowers.	" " "	28	+++

The flannel vesting alone and with mineral oil gave no reaction.

adjacent to (x). The patches were removed after 28 hours because of considerable irritation and the nausea occasioned by the all-pervading smell of the oil. The reactions were as follows :

(x) *Essential oil, free from acids.*—The test area was clearly outlined, uniformly red, and slightly swollen. No vesicles.

(y) *Steam-distilled flowers (moist)*.—Reaction similar to that of (x), but slightly more swollen. No vesicles.

Each represented a positive reaction of moderate intensity. Both lesions were still clearly visible on 27 August, with definite outlines. At the time of removal of the patches the irritation due to (y) appeared to be somewhat greater than that due to (x), but the latter caused more irritation during the period of recovery.

It is clear that while the oil obtained by steam distillation, and free from acid constituents, was capable of causing dermatitis, the residual flowers still contained an active agent.

The intensities of the reactions obtained, evaluated at the time of removal of the patches by the assignation of plus signs, are summarized in Table I.

General Constitutional Reaction.

On 25 June, in an attempt to obtain information in a minimum of time, eight patches in all were applied, four to each thigh. It was anticipated that not more than two of these would give an intense reaction, and two such patches had previously been carried without general ill-effect. Actually, however, all the patches gave reactions of varying intensities, and by the morning of 27 June, when the patches were removed, the irritation had become acute. A smarting of the face was noticed, with puffiness and redness under the right eye.

Later, persistent faintness and shivering were experienced and at noon J. T. Martin retired to bed. At this time the body temperature was 97.1° F. On examination by K. H. C. Hester, at 3.30 p.m. the temperature was 98.4° and the pulse-rate 100 per minute. The shivering continued, the face appeared rather paler than usual and the skin was warm and very moist.

Five hours later the temperature had risen to 99.4° F. and the pulse-rate was still 100. The face was now more flushed; the skin was warm and moist and exhibited well-marked dermatographism. The tongue was rather furred but moist, the fauces were clear, and there were no abnormal signs in the circulatory, respiratory, abdominal and nervous systems. Aspirin, gr. x. was taken at night, and there was later fairly profuse sweating. At midday on 28 June the body temperature was normal and the pulse-rate 80. The face still smarted, but the eye had recovered. Six hours later

the skin of the upper arm still exhibited marked dermatographism. Recovery was complete by the morning of 30 June.

DISCUSSION OF RESULTS.

Throughout the investigation an attempt was made to keep the amounts of the materials tested as constant as possible. Strictly comparable tests were not possible owing to the differences in physical states of the products examined, resins achieving a more intimate contact with the skin than crystalline masses.

Certain definite conclusions, however, are possible. Early tests indicated that the pyrethrins could not be regarded as being responsible for the dermatitis, and definite evidence substantiating this was obtained when the pyrethrin concentrate was tested. The period allowed for the pyrethrins to act was 27 hours, but the intense reaction produced by the volatile oil over a similar period (test (w)) indicated that this was long enough to produce a definite reaction if an agent responsible for the dermatitis was present.

The intensity of the reaction given by the colourless extract and the absence of an intense reaction when the fatty products were tested disposed of the possibility that the pigments, fats and probably the free acids could be responsible. In addition, it was unlikely that protein material would be included in the petroleum ether extract of the flowers, and the possibility of a protein being the only causal agent was thus ruled out from the commencement of the investigation.

The intense reaction given by the volatile oil, and the positive, though less intense, reaction given by the oil after the removal of free acids, were of great interest. An important causal agent is present in the volatile oil, and, moreover, must be thermostable at 100° C. That it is the only responsible constituent is not so certain, as the powdered flowers after subjection to steam distillation were still active. This may have been due, however, to incomplete removal of the oil (the flowers when tested still smelt of pyrethrum) or to the presence of other active compounds. In this connection, the reactions given by the crystalline material separating directly from a petroleum ether extract, and recrystallized from alcohol (test (s)), and possibly by the crystalline fraction of the extract insoluble in acetic acid and also recovered from alcoholic solution

(test (u)), may be of significance. There still remains, in addition, the possibility of the presence of a water-soluble active agent. The more intense reaction given by moistened flowers than by air-dried flowers may be a reflection of this, or merely due to more intimate contact with the skin. Further work is required to determine to what extent the essential oil and possibly other substances responsible for dermatitis may be removed from extracts of pyrethrum without serious loss of the pyrethrins, and this we hope to do. The desirability of further examination of the volatile oil is also indicated.

It is of interest to record that although the dermatitis produced was in some cases acute, at no stage in the investigation were there any disturbances in the respiratory tract, supporting the views of Feinberg and Sequeira that the agent responsible for dermatitis differs from that causing respiratory troubles.

It has been assumed that wind-blown pollen has been responsible for the ability of pyrethrum to cause dermatitis at a distance. The almost negative reaction given by pollen when tested disposes of this possibility. On the other hand, this effect may readily be explained by the strong dermatitis-producing property of the volatile oil.

We are indebted to Dr. F. Tattersfield for his interest and assistance in the separation of the pollen and the dissection of whole flowers, and to Dr. A. M. H. Gray, Prof. P. A. Buxton and Dr. J. Henderson Smith for their interest in the work.

SUMMARY.

1. Patch tests of powdered and dissected pyrethrum flowers have been carried out. The causal agent, or agents, of the dermatitis appears to be concentrated in the lower parts of the flower-head. Powdered flowers moistened with water gave a more intense reaction than the air-dried ground flowers. Pollen separated from the flowers gave only a slight reaction.

2. Ether and petroleum ether extracts of the flowers have been fractionated and a number of products tested for their ability to produce dermatitis. A colourless extract, obtained by petroleum ether extraction of the flowers mixed with charcoal, gave an intense reaction. The pyrethrins, tested in a concentrate containing 93%.

of apparent pyrethrins as determined by a modified Seil method, are shown to be devoid of dermatitis-producing properties.

3. The volatile oil, obtained by steam distillation of powdered flowers, is shown to be highly active. The oil after the extraction of a volatile acid fraction with alkali gave a less intense reaction, while the steam distilled flowers also gave a positive reaction. The possibility that crystalline constituents of the petroleum ether extract of the flowers are contributory causal agents of the dermatitis is indicated.

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